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(54) Title: **ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV-1 GAG, POL, NEF AND MODIFICATIONS**

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING  
CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

## 5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S.  
provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2  
(serial number unassigned), filed September 15, 2000, March 27, 2001, and  
September 7, 2001, respectively.

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## STATEMENT REGARDING FEDERALLY-SPONSORED R&amp;D

Not Applicable

## REFERENCE TO MICROFICHE APPENDIX

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Not Applicable

## FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first  
generation adenovirus vaccines found to exhibit enhanced growth properties and  
greater cellular-mediated immunity as compared to other replication-deficient vectors.  
The invention also relates to the associated first generation adenoviral vectors  
described herein, which, through the incorporation of additional 5' adenovirus  
sequence, enhance large scale production efficiency of the recombinant, replication-  
defective adenovirus described herein. Another aspect of the instant invention is the  
surprising discovery that the intron A portion of the human cytomegalovirus (hCMV)  
promoter constitutes a region of instability in adenoviral vector constructs. Removal  
of this region from adenoviral expression constructs results in greatly improved vector  
stability. Therefore, improved vectors expressing a transgene under the control of an  
intron A-deleted CMV promoter constitute a further aspect of this invention. These  
adenoviral vectors are useful for generating recombinant adenovirus vaccines against  
human immunodeficiency virus (HIV). In particular, the first generation adenovirus  
vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-  
1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide  
pharmaceutical products, and biologically active modifications thereof. Host  
administration of the recombinant, replication-deficient adenovirus vaccines described  
herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

#### BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNase H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNase H (RNase, p15) activities.



The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8<sup>+</sup> T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8<sup>+</sup> T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4<sup>+</sup> T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see, e.g.*, Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose single and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag*, *pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

#### SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH<sub>2</sub>-terminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5' region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine  
5 vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced  
10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more  
15 important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a  
20 region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or  
25 biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-  
30 3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1  
35 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises:

5 a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene

10 expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral

15 vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested

20 recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6<sup>®</sup> cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material

25 which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual

30 an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto,

35 base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5 In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to  
10 mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response  
15 upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

20 To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine  
25 plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then  
30 a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In  
35 these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of



differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

5           The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not  
10       limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen  
15       with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of  
20       such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

          The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be  
25       ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25)  
30       within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second  
35       harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

5 It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

10 It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

15 It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair  
20 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a  
25 polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV  
30 infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a  
35 single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective.

5 They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10 "s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

15 "Ad5-FLgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

20 "Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

25 "Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

30 "Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

35 "Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as  
5 exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to  
10 the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the  
15 E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct  
20 also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

25 "pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

30 "pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

35 "pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdeIE1sp1A" or "MRKpdeIE1(Pac/pIX/pack450)" or

5 "MRKpdeIE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1  
10 antiparallel) orientation)

"MRKpdeIE1(Pac/pIX/pack450)+CMVmin+bGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has  
15 been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IAPol and G2A,LLAA nef genes directly into.

20 "MRKpdeIE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdeIE1 shuttle +hCMV-FL-gag-BGHpA"

25 "MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or  
30 "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt)" is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the *Bgl*II site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a  
35 plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

5 "MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

10 "pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns  
15 and/or V1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as  
20 "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

#### BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

30 Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

35 Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

5 Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

15 Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

20 Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

25 Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

30 Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*I and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

35 Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);



(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5        Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed  
10        herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences  
15        through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH<sub>2</sub>-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate  
20        consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding  
25        sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as  
30        underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174  
35        and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "\*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

5        Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

10       Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

15       Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

20       Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

25       Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular  $\gamma$ IFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti- $\gamma$ IFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and  $\gamma$ IFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+ $\gamma$ IFN+ and CD4+ $\gamma$ IFN+, respectively.

35       Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IAPol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IAPol fusion frame.

5

## DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained its correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

35

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6<sup>®</sup> cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as

5 MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both

10 constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S.

15 Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon

20 optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a

25 construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs

30 disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact

35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt



Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH<sub>2</sub>-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration  
5 increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include  
10 any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef  
15 constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this  
20 plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses  
25 the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression  
30 regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can  
35 be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a  
5 nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of  
10 interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine,  
15 especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 *pol* open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a  
20 human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and  
25 essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or  
30 biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S.  
35 Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+).

Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficacious adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors  
5 not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6<sup>®</sup> cells and virus is produced. The infected cells and media were  
15 harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6<sup>®</sup>. Both these cell lines express the adenoviral E1 gene product. PER.C6<sup>®</sup> is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby  
20 incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6<sup>®</sup>,  
25 from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be  
35 used as human anti-HIV vaccines, and are capable of inducing immune responses.



Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as  
5 buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM  $MgCl_2$ ; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably  
10 about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM  $MgCl_2$ , 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface.  
15 It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene  
20 product. In general, an immunologically or prophylactically effective dose of  $1 \times 10^7$  to  $1 \times 10^{12}$  particles and preferably about  $1 \times 10^{10}$  to  $1 \times 10^{11}$  particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also  
25 contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine  
30 compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile  
35 saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

#### EXAMPLE 1

##### Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVII<sub>ns</sub>HIVgag was used as the starting material to amplify the hCMV promoter. PVI<sub>ns</sub>HIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *MscI* site of the hCMV promoter and a 3' primer (designed to contain the *Bgl*<sup>III</sup> recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *MscI* and *Bgl*<sup>III</sup>. This fragment was then cloned back into the original GMP grade pVI<sub>ns</sub>HIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *MscI* and *Bgl*<sup>III</sup> digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pVI<sub>ns</sub>HIVgag vector backbone. This vector is designated pVII<sub>ns</sub>CMV(no intron).

The FLgag gene was excised from pVI<sub>ns</sub>HIVgag using *Bgl*<sup>III</sup> digestion and the 1,526 bp gene was gel purified and cloned into pVI<sub>ns</sub>CMV(no intron) at the *Bgl*<sup>III</sup> site. Colonies were screened using *Sma*<sup>I</sup> restriction enzymes to identify clones that carried the FLgag gene in the correct orientation. This plasmid, designated pVI<sub>ns</sub>CMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)<sub>n</sub>, and (T)<sub>n</sub>; respectively) underlined:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTGTGTG  
(SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

## EXAMPLE 2

### Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	$\mu\text{g gag}/10^6 \text{ COS cells}/5\mu\text{g DNA}/48 \text{ hr}$
HIVFL-gagPR9901 <sup>a</sup>	10.8
PV1Ins-hCMV-FLgag-bGHpA <sup>b</sup>	16.6
pV1Ins-hCMV-FLgag-SPA <sup>b,c</sup>	12.0

<sup>a</sup> GMP grade pV1Ins-hCMVintronA-FLgag-bGHpA.

5 <sup>b</sup> New plasmid constructions that have the intron A portion removed from the hCMV promoter.

<sup>c</sup> In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

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### EXAMPLE 3

#### Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1InsCMV(no intron)-FLgag-bGHpA and pV1InsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above  
 15 possessing the intron A portion of the CMV promoter, pV1InsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which  
 20 are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20  $\mu\text{g}$  and 200  $\mu\text{g}$ .

## EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA <sup>a</sup> Promoter/terminator	Dose, ug <sup>b</sup>	Anti-p24 Titers (3 Wk PD1) <sup>c</sup>			SFC/10 <sup>6</sup> Cells (4 Wk PD1) <sup>d</sup>		
		GMT	+SE	-SE	Media	gag197-205	p24
HIVFL-gagPR9901 (GMP grade)	200	12800	4652	3412	2(2)	129(19)	30(11)
	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV- FL-gag-bGHpA	200	11143	2831	2257	0	98(5)	12(6)
	20	7352	2808	2032	0	73(9)	11(6)
pV1Jns-hCMV- FL-gag-SPA	200	16890	5815	4326	1(1)	94(4)	26(7)
	20	5971	5361	2825	0	85(17)	38(10)
Naïve	0	123	50	36	0	0	0

<sup>a</sup>in PBS<sup>b</sup>i.m. Injections into both quads, 50 µL per quad<sup>c</sup>n=10; GMT, geometric mean titer; SE, standard. error<sup>d</sup>n=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

## Construction of the Modified Shuttle Vector -"MRKpdelE1 Shuttle"

- The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:
- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
  - (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
  - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6<sup>®</sup> cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

## EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions ) and pAdHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdeIE1 shuttle) with *Pac*I and *Bst*Z1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *Cla*I linearized pAdHVO (E3- adenovector) or *Cla*I linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained *Cla*I , *Bam*HI, *Xho* I, *Eco*RV, *Hind*III, *Sal* I, and *Bgl* II sites. This MCS was replaced with a new MCS containing *Not* I, *Cla* I, *Eco*RV and *Asc* I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and *pIX* gene.

## EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac*I to remove the vector backbone) and subsequently labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

#### EXAMPLE 7

##### Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *Hind*III (and *Pac*I to remove the vector backbone) and then labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly



compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

#### EXAMPLE 8

##### Construction of the new shuttle vector containing modified gag transgene – “MRKpdeIE1-CMV(no intron)-FLgag-bGHpA”

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *MscI* overnight and then digested with *SfiI* for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdeIE1 shuttle) was linearized by digestion with *EcoRV*, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdeIE1 shuttle vector.

#### EXAMPLE 9

##### Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdeIE1-CMV(no intron)-FLgag-bGHpA, was digested with *PacI*. The reaction mixture was digested with *BsfZ171*. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *ClaI* overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH<sub>2</sub>O. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *BstEII* which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

## EXAMPLE 10

### Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

## EXAMPLE 11

Virus generation of an enhanced adenoviral construct – “MRK Ad5 HIV-1 gag”

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *Pac1* to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6<sup>®</sup> cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6<sup>®</sup> cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6<sup>®</sup> cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *HindIII* and radioactively labeled with [<sup>33</sup>P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pac1/HindIII* prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

## EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11.

Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *HindIII* digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:  
Amplification Ratios Based on AEX and QPA Analysis of  
Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

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\* This estimation is based on the clinical lot growth characteristics at Passage 12.

### EXAMPLE 13

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#### Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

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Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32,905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

- 5           Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type
- 10   Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

**Table 5A:** Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

### MRKAd5gag rep1

	Xv (10 <sup>6</sup> cells/ml) Infection	Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 <sup>6</sup> vp/ml culture	Titer 10 <sup>6</sup> vp/cell	QPA 10 <sup>6</sup> TCID <sub>50</sub> /ml	Ratio AEX:QPA	Amplification Ratio 470 (MOI = 125)	AEX Internal Control
P4	1.49, 81%	0.58, 50%	44	45	6.7	5.9	1.72	50	170	
P5	1.38, 83%	0.55, 47%	48	49	6.7	4.9	1.38	49	200	
P6	1.04, 84%	0.88, 77%	47	48	5.8	5.6	1.42	41	50	
P7	1.50, 84%	0.95, 61%	49.5	50	3.9	1.4	0.97	40	170	
P7	1.09, 97%	0.76, 65%	50	52	5.2	4.7	1.70	81	310	
P8	1.03, 94%	0.85, 64%	47.5	54	9.0	6.7	1.10	82	175	3.12
P9	0.89, 95%	0.99, 73%	47.5	56	4.4	4.9	1.03	43	100	2.84
P10	1.09, 91%	1.06, 66%	47.5	58	3.0	2.8	1.18	28	270	2.70
P11	1.18, 88%	0.88, 65%	47	60	3.6	3.0	1.15	31	260	2.60
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	210	3.18
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	52	160	3.28
P14	1.94, 82%	0.88, 67%	46	53	6.5	4.4			250	2.91
P15	0.97, 86%	0.64, 66%	47	47	6.9	7.1				2.91

**Table 5B:** Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

### MRKHVE3

	Xv (10 <sup>6</sup> cells/ml) Infection	Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 <sup>6</sup> vp/ml culture	Titer 10 <sup>6</sup> vp/cell	QPA 10 <sup>6</sup> TCID <sub>50</sub> /ml	Ratio AEX:QPA	Amplification Ratio 300 (MOI = 125)	AEX Internal Control
P4	1.10, 87%	1.28, 76%	49	54	4.1	3.8	1.70	25	170	
P5	0.92, 89%	1.18, 77%	47	48	4.3	4.7	1.24	35	30	
P6	1.55, 88%	1.28, 76%	49.5	50	1.2	0.8	0.58	21	110	
P6	1.09, 87%	1.11, 81%	49	52	4.0	3.6	1.18	34	75	3.12
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	25	2.84
P8	0.98, 88%	1.41, 63%	48	56	2.1	2.1	0.47	45	80	2.70
P9	1.20, 89%	1.28, 81%	47.5	58	0.8	0.7	0.29	28	270	2.60
P10	0.99, 82%	1.53, 66%	47	60	2.3	2.3	0.43	53	90	2.70
P11	1.07, 96%	1.25, 63%	48	47	2.7	2.5	0.41	66	280	2.86
P12	0.80, 91%	1.14, 80%	49.5	49	6.9	7.4	0.48	123	110	3.18
P13	1.86, 85%	1.14, 85%	45.5	53	5.8	3.0			350	3.28
P14	0.97, 96%	1.03, 98%	48.5	47	9.4	8.7			218	2.91
P15	0.87, 99%	0.87, 89%	49.5	49	5.3	6.1				2.78



Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

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### MRKAd5gag(E3-)

	Xv (10 <sup>6</sup> cells/ml)	Viability (%)	Harvest Time	Cell Passage	Titer	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	h.p.i.	Number	10 <sup>6</sup> vp/ml culture	10 <sup>6</sup> vp/cell	10 <sup>6</sup> TCID <sub>50</sub> /ml	AEX:QPA	Ratio	Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.92	20	100	
P5	1.16, 82%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 85%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P8	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	190	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.84
P10	0.99, 82%	0.80, 62%	48.5	60	3.2	3.2	0.68	47	115	2.70
P11	1.07, 96%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.70
P12	0.80, 81%	0.67, 59%	50	49	6.1	6.4	0.72	71	230	2.85
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			185	2.60
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.18
P15	0.87, 89%	0.84, 66%	49	49	4.8	5.5			196	3.18

### EXAMPLE 14

#### Gag Expression Analysis of the Novel Constructs

*In vitro* gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHPA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

### EXAMPLE 15

#### Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag ( $10^7$  and  $10^9$  vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

Viral Vectors <sup>a</sup>	$\mu\text{g gag}/4.8 \times 10^5 \text{ COS}/10^8 \text{ parts}/48\text{hr}$
MRKAd5gag <sup>b</sup>	1.40
Clinical lot Ad5gag <sup>c</sup>	1.28
Research lot Ad5gag <sup>d</sup>	1.32
MCMVFL-gagbGHpA <sup>e</sup>	0.42

<sup>a</sup>  $A_{260\text{nm}}$  absorbance readings taken for viral particle determinations.

<sup>b</sup> MRKAd5gag was produced in serum free conditions and purified at P5.

<sup>c</sup> Clinical lot# Ad5gagFN0001

<sup>d</sup> Research Ad5FLgag lot# 6399

<sup>e</sup> mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

**Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).**

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	<sup>a</sup> MRKAd5gag	10 <sup>7</sup>	25600	5877	4780
2	"	10 <sup>9</sup>	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10 <sup>7</sup>	7352	2077	1620
4	"	10 <sup>9</sup>	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10 <sup>7</sup>	12800	9905	236
6	"	10 <sup>9</sup>	310419	99181	75165
7	<sup>b</sup> mCMV FL-gag bGHpA [E3+] →	10 <sup>7</sup>	44572	23504	15389
8	"	10 <sup>9</sup>	941014	239068	190636
9	<sup>c</sup> hCMV FL-gag bGHpA [E3-] ←	10 <sup>7</sup>	3676	934	745
10	"	10 <sup>9</sup>	117627	17491	15227
11	research lot hCMV IntronA FL-gag bGHpA [E3-] <-	10 <sup>6</sup>	528	262	175
12	"	10 <sup>7</sup>	14703	5274	3882
13	"	10 <sup>8</sup>	58813	14942	11915
14	"	10 <sup>9</sup>	204800	53232	42250
15	clinical lot hCMV IntronA FL-gag bGHpA [E3-] <-	10 <sup>6</sup>	230	82	61
16	"	10 <sup>7</sup>	4222	3405	1138
17	"	10 <sup>8</sup>	19401	3939	3274
18	"	10 <sup>9</sup>	89144	25187	19639
19	Naïve	none	93	7	6

\*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro

Vaccination: T. Toner, Q. Su

Assay: M. Chen

<sup>a</sup>The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The same lot of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

<sup>b</sup>The same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) was used here.

<sup>c</sup>This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10<sup>6</sup>7 dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

## EXAMPLE 16

### Comparison of Humoral and Cellular Responses Towards the Original Ad-gag

#### Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

- 5 Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10<sup>11</sup> vp and 10<sup>9</sup> vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-  
10 gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

- peripheral blood assmumarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after
- 5 CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gag <sup>0</sup> , 10 <sup>11</sup> vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10 <sup>9</sup> vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag <sup>0</sup> , Clinical Lot, 10 <sup>11</sup> vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10 <sup>9</sup> vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
MRKAd5gag(hCMV, bGHpA, E3+)								
original Ad5gag vector (hCMV/intron A, bGHpA, E3-), lot#FN0001								
ND, not determined								

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4<sup>+</sup> T cells.

Grp #	Vaccination T=0,4,25 wks	Monkey ID	T=4 Wk		T=6 Wk		T=11 Wk		T=16 Wk		T=25 Wk		T=28 Wk	
			Media <sup>a</sup>	Gag H <sup>b</sup>	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H
1	MRKAd5gag 10 <sup>9</sup> vp	97N010	6	89	0	385	0	1058	0	1174	3	775	4	1074
		97N010(CD4-)	4	38			3	993			0	76	0	594
		97N116	1	398	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	11	676			0	593			0	184	0	666
		98X007	10	579	0	1304	3	2193	1	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2675			0	1656	0	1278
2	MRKAd5gag 10 <sup>9</sup> vp	97N120	5	275	1	249	4	141	4	119	9	206	4	219
		97N120(CD4-)	11	170			0	85			0	75	1	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148			0	285			ND	ND	0	625
		98X008	4	388	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696			0	1175			0	391	4	648
3	Ad5gag clinical lot 10 <sup>9</sup> vp	97X001	0	281	1	485	0	817	0	1220	1	894	0	1858
		97X001(CD4-)	10	283			3	996			0	1010	0	1123
		97N146	3	150	1	465	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133			0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	1748
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Ad5gag clinical lot 10 <sup>9</sup> vp	97N020	3	30	1	101	0	66	0	36	0	26	0	41
		97N020(CD4-)	10	29			0	15			0	1	0	16
		97X003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	85	3	54	1	34	0	18	0	20	1	121
		98X012(CD4-)	11	70			0	11			0	8	0	41
5	Native	96R041	8	8	1	1	0	0	0	0	0	0	1	0
		053F	14	18	5	18	0	14	0	15	0	15	24	9

Based on either 4x10<sup>6</sup> or 2x10<sup>6</sup> cells per well (depending on spot density)

ND, not determined

<sup>a</sup>Mock or no peptide control

<sup>b</sup>Pool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

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The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10<sup>9</sup> vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

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#### EXAMPLE 17

#### CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

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The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprises codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized)") wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC  
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC  
 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG  
 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC  
 CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC  
 5 TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC  
 AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC  
 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC  
 CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT  
 GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC  
 10 ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC  
 CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT  
 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCTCCC AAATCTACCC TGGCATCAAG  
 GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG  
 ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT  
 15 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC  
 CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC  
 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC  
 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG  
 GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG  
 20 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTTGT  
 GGGGCTGAGA CCTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT  
 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG  
 AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT  
 GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT  
 25 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG  
 GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC  
 ATCAGGAAGG TGCTGTTTCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC  
 CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG  
 ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC  
 30 TGCTCCCCCTG GCATCTGGCA GCTGGACTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG  
 GCTGTGCATG TGGCCTCCGG CTACATGTAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG  
 GAGACTGCCT ACTTCTTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT  
 GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC  
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGG GTCCATGAAC  
 35 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT  
 GTGCAGATGG CTGTGTTTCAT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG  
 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCTGTGG  
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT  
 GACATCAAGG TGGTGCCCGAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG  
 5 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ  
 ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID  
 NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro  
 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys  
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys  
 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala  
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg  
 Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile  
 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys  
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile  
 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala  
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln  
 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly  
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg  
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln  
 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys  
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile  
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr  
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu  
 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr  
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln  
 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys  
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys  
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile  
 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp  
 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu  
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala



Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly  
 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu  
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn  
 Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro  
 5 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile  
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys  
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys  
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro  
 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys  
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln  
 Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His  
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly  
 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val  
 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro  
 Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu  
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr  
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly  
 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr  
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn  
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro  
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn  
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp  
 Glu Asp (SEQ ID NO:2) .

The present invention especially relates to an adenoviral vector vaccine which  
 comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to  
 deletion of the portion of the wild type sequence encoding the protease activity, a  
 30 combination of active site residue mutations are introduced which are deleterious to  
 HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present  
 invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein  
 the construct is devoid of DNA sequences encoding any PR activity, as well as  
 containing a mutation(s) which at least partially, and preferably substantially,  
 35 abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part  
 and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

	<u>wt aa</u>	<u>aa residue</u>	<u>mutant aa</u>	<u>enzyme function</u>
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

```

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGAATTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
15 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGAAGTGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
25 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGAAGTACAC CACCAACCAG
AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCTGCCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
35 ATCAGGAAGG TGCTGTTCCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

```

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC  
 TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG  
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG  
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT  
 5 GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC  
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC  
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT  
 GTGCAGATGG CTGTGTTTCA CCACAATTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC  
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG  
 10 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG  
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT  
 GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG  
 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID  
 NO:3) .

15 In order to produce the IA-pol-based adenoviral vaccines of the present  
 invention, inactivation of the enzymatic functions was achieved by replacing a total of  
 nine active site residues from the enzyme subunits with alanine side-chains. As  
 shown in Table 1, all residues that comprise the catalytic triad of the polymerase,  
 namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues  
 20 (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.*  
 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445,  
 Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this  
 IA Pol construct), with each residue being substituted for an Ala residue, respectively  
 (Davies, et al., 1991, *Science* 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-  
 25 314; Mizrahi, et al., 1990, *Nucl. Acids. Res.* 18: pp. 5359-5353). HIV pol integrase  
 function was abolished through three mutations at Asp626, Asp678 and Glu714.  
 Again, each of these residues has been substituted with an Ala residue (Wiskerchen,  
 et al., 1995, *J. Virol.* 69: 376-386; Leavitt, et al., 1993, *J. Biol. Chem.* 268: 2113-  
 2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene.  
 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and  
 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro  
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys  
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys  
 35 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala  
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile  
 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys  
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile  
 5 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala  
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln  
 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly  
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg  
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln  
 10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys  
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile  
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr  
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu  
 15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr  
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln  
 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys  
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys  
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile  
 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp  
 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu  
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala  
 Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly  
 25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala  
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn  
 Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro  
 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile  
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
 30 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys  
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys  
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro  
 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys  
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln  
 35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His  
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val  
 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro  
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu  
 5 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr  
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly  
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr  
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn  
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro  
 10 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn  
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp  
 Glu Asp (SEQ ID NO:4) .

As noted above, it will be understood that any combination of the mutations  
 15 disclosed above may be suitable and therefore be utilized as an IA-pol-based  
 adenoviral HIV vaccine of the present invention, either when administered alone or in  
 a combined modality regime and/or a prime-boost regimen. For example, it may be  
 possible to mutate only 2 of the 3 residues within the respective reverse transcriptase,  
 RNase-H, and integrase coding regions while still abolishing these enzymatic  
 20 activities. However, the IA-pol construct described above and disclosed as SEQ ID  
 NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also  
 preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1  
 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal  
 25 peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide  
 such as is found in highly expressed mammalian proteins such as immunoglobulin  
 leader peptides. Any functional leader peptide may be tested for efficacy. However,  
 a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown  
 herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein  
 30 the pol coding region or a portion thereof is operatively linked to a leader peptide,  
 preferably a leader peptide from human tPA. In other words, a codon optimized  
 HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide  
 at the amino terminal portion of the protein, which may effect cellular trafficking and  
 hence, immunogenicity of the expressed protein within the host cell. As noted in  
 35 Figure 16A-B, a DNA vector which may be utilized to practice the present invention  
 may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGRLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region ( herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

25 GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT  
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCCTGTGAA  
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT  
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG  
CCCCGAGAAC CCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG  
30 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA  
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT  
GGGGGATGCC TACTTCTCTG TGCCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCPTCAC  
CATCCCCCTC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA  
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT  
35 CAGGAAGCAG AACCCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC  
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG  
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATTT GTGCTGCCTG AGAAGGACTC  
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA  
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA  
 5 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA  
 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA  
 GCAGGGCCAG GGCCAGTGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC  
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGAAGTGGG  
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT  
 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT  
 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA  
 GGAGCCCATTT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA  
 GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA  
 CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT  
 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA  
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT  
 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT  
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA  
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT  
 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA  
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGACCCACC TGGAGGGCAA  
 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC  
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA  
 GACCATCCAC ACTGACAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG  
 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCAGTCCC AGGGGGTGGT  
 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA  
 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT  
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA  
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG  
 30 GAACCCCTG TGAAGGGGCC CTGCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT  
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA  
 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC  
 GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ  
 35 ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:  
 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly



Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile  
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val  
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile  
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu  
 5 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr  
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln  
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys  
 Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser  
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro  
 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu  
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr  
 Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr  
 Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln  
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly  
 15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp  
 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val  
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val  
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg  
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile  
 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile  
 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu  
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile  
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met  
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln  
 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe  
 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr  
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro  
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala  
 Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly  
 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu  
 Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala  
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr  
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu  
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu  
 35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp  
 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala  
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val  
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln  
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu  
 5 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu  
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu  
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn  
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala  
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly  
 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val  
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe  
 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly  
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu  
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp  
 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly  
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro  
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly  
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6) .

The present invention also relates to a codon optimized HIV-1 Pol mutant  
 20 contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4)  
 which comprises a leader peptide at the amino terminal portion of the protein, which  
 may effect cellular trafficking and hence, immunogenicity of the expressed protein  
 within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in  
 the above paragraphs is suitable for fusion downstream of a leader peptide, such as a  
 25 leader peptide including but not limited to the human tPA leader sequence. Therefore,  
 any such leader peptide-based HIV-1 pol mutant construct may include but is not  
 limited to a mutated DNA molecule which effectively alters the catalytic activity of  
 the RT, RNase and/or IN region of the expressed protein, resulting in at least  
 substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN  
 30 functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a  
 leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the  
 Pol coding region which effectively abolishes RT, RNase H and IN activity. An  
 especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at  
 least one point mutation which alters the active site and catalytic activity within the  
 35 RT, RNase H and IN domains of Pol, such that each activity is at least substantially  
 abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed  
 5 herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open  
 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT  
 CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCC ATTGAGACTG TGCTGTGAA  
 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT  
 CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG  
 CCCCAGAAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG  
 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA  
 GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT  
 20 GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC  
 CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA  
 GGGCTGGAAG GGCTCCCTG CCATCTTCCA GTCTCCATG ACCAAGATCC TGGAGCCCTT  
 CAGGAAGCAG AACCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC  
 TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG  
 25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG  
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC  
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA  
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA  
 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA  
 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA  
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC  
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGA CTGAGGC  
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCA AGTTCAAGCT  
 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT  
 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA  
 GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA  
 CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT  
 GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA  
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT  
 5 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT  
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA  
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT  
 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA  
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA  
 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC  
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA  
 GACCATCCAC ACTGCCAATG GCTCCAACIT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG  
 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCAGTCCC AGGGGGTGGT  
 GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA  
 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT  
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA  
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG  
 GAACCCCTG TGAAGGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT  
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA  
 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC  
 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile  
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val  
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile  
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu  
 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr  
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln  
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys  
 Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser  
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro  
 35 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu  
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr  
 Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln  
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly  
 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp  
 5 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val  
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val  
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg  
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile  
 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile  
 10 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu  
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile  
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met  
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln  
 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe  
 15 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr  
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro  
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala  
 Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly  
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu  
 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala  
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr  
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu  
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu  
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp  
 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile  
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala  
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val  
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln  
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu  
 30 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu  
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu  
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn  
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala  
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly  
 35 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val  
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly  
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu  
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp  
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly  
 5 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro  
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly  
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

### EXAMPLE 18

#### 10 CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed  
 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein  
 20 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH<sub>2</sub>-terminus of the HIV-1 Nef  
 25 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and  
 30 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA  
 35 molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

- 5 As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jfr1  
 10 nef gene is disclosed herein as SEQ ID NO:9, as shown herein:  
 GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA  
 GGGAGAGGAT GAGGAGGGCC GAGCCC GCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG  
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA  
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG  
 15 GCTTCCCCGT GAGGCCCCAG GTGCCCTTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC  
 TGTCCTACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC  
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT  
 ACACCCCCCG CCCCGGCATC AGGTTCCTCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC  
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACCTGC CTGCTGCACC  
 20 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT  
 CCAAGCTGGC CTTCACCCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT  
 AAAGCCCCGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG),  
 Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG);  
 25 Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG),  
 Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian  
 (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby  
 incorporated by reference. See also Figure 19A-B for a comparison of wild type vs.  
 codon optimized nucleotides comprising the open reading frame of HIV-Nef.

30 The open reading frame for SEQ ID NO:9 above comprises an initiating  
 methionine-residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides  
 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid  
 HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine  
 vector. The 216 amino acid HIV-1 Nef (jfr1) protein is disclosed herein as SEQ ID  
 35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg  
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu  
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp  
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val  
 5 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp  
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His  
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln  
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg  
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro  
 10 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His  
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu  
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu  
 His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the  
 15 inner surface of the host cell plasma membrane through myristylation of Gly-2  
 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions  
 have been elucidated, it has become clear that correct trafficking of Nef to the inner  
 plasma membrane promotes viral replication by altering the host intracellular  
 environment to facilitate the early phase of the HIV-1 life cycle and by increasing the  
 20 infectivity of progeny viral particles. In one aspect of the invention regarding  
 codon-optimized, protein-modified polypeptides, the nef-encoding region of the  
 adenovirus vector of the present invention is modified to contain a nucleotide  
 sequence which encodes a heterologous leader peptide such that the amino terminal  
 region of the expressed protein will contain the leader peptide. The diversity of  
 25 function that typifies eukaryotic cells depends upon the structural differentiation of  
 their membrane boundaries. To generate and maintain these structures, proteins must  
 be transported from their site of synthesis in the endoplasmic reticulum to  
 predetermined destinations throughout the cell. This requires that the trafficking  
 proteins display sorting signals that are recognized by the molecular machinery  
 30 responsible for route selection located at the access points to the main trafficking  
 pathways. Sorting decisions for most proteins need to be made only once as they  
 traverse their biosynthetic pathways since their final destination, the cellular location  
 at which they perform their function, becomes their permanent residence.  
 Maintenance of intracellular integrity depends in part on the selective sorting and  
 35 accurate transport of proteins to their correct destinations. Defined sequence motifs  
 exist in proteins which can act as 'address labels'. A number of sorting signals have



been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

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CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCCAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCTTGCTGC ACCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCT ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCC
(SEQ ID NO:11).

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The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfr1) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro  
Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala  
10 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val  
Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala  
Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu  
Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr  
Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu  
15 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp  
Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro  
Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu  
Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn  
Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu  
20 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His  
Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).  
Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfr1 isolate wherein the codons are optimized for  
25 expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jfr1 nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13,  
35 as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA  
 GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG  
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA  
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG  
 5 GCTTCCCCGT GAGGCCCCAG GTGCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC  
 TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC  
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT  
 ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC  
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACGTC GCCGCCACC  
 10 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTGACT  
 CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT  
 AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val  
 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg  
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu  
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp  
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val  
 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp  
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His  
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln  
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg  
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro  
 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His  
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu  
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu  
 His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

30 An additional embodiment of the present invention relates to another DNA  
 molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation  
 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide.  
 This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which  
 encodes a Nef protein containing a tPA leader sequence fused to amino acid residue  
 6-216 of HIV-1 Nef (jfr1), wherein Leu-174 and Leu-175 are substituted with Ala-174  
 35 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT  
 TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG  
 5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG  
 CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC  
 CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC  
 CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA  
 CTTCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT  
 10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC  
 CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGG  
 GCCCCAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCATGTC  
 CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCTG ACTCCAAGCT  
 GGCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGGTAC TACAAGGACT GCTAAAGCCC  
 15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro  
 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala  
 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val  
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala  
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu  
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr  
 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu  
 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp  
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro  
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu  
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn  
 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu  
 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His  
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence,  
 regardless of codon usage, which expresses a wild type or modified Nef protein as  
 35 described herein, including but not limited to modified Nef proteins which comprise a  
 deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

## EXAMPLE 19

### MRKAd5Pol Construction and Virus Rescue

*Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)-*Cla*I (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the *Pac*I site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with *Bgl* II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*III site. The clones were checked for the correct orientation of the gene by using restriction enzymes *Dra*III/*Nor*I. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *Pac*I and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)*Cla*I. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

*Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAd5pol was digested with restriction enzyme *Pac*I (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pac*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6<sup>®</sup> cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

## EXAMPLE 20

### MRKAd5Nef Construction and Virus Rescue

*Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac1* site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl11* site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl11* releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

MRKpdeIE1+CMVmin+BGHPA(str.) shuttle vector at the *Bgl11* site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca1*. A positive clone was isolated and named MRKpdeIE1hCMVminFL-nefBGHPA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdeIE1hCMVminFL-nefBGHPA(s) was digested with restriction enzymes *Pac1* and *Bst1107 I* (or its isoschizomer, *BstZ107 I*) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla1* digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdeIE1hCMVminFL-nefBGHPA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

*Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme *Pac1* (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech



Inc.). *Pac1* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6<sup>®</sup> cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at  $\leq -60^{\circ}\text{C}$ . This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

### EXAMPLE 21

#### Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA ATG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II) Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHPA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with *Asc* I and *Bgl* II to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

*Bgl* II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by *Bgl* II digestion.

#### EXAMPLE 22

##### 5 Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*I and *Bst*Z110I digestion of each shuttle vector was performed and each specific transgene  
10 fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla* I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently  
15 prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

#### EXAMPLE 23

##### Construction of hCMV-tpa-nef (LLAA) Adenovector

20 The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *Bam*HI, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-  
25 bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca* I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac* I and *Bst* Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial  
30 homologous recombination techniques.

#### EXAMPLE 24

##### Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c  
35 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either  $10^7$  vp and  $10^9$  vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10<sup>7</sup> vp and 10<sup>9</sup> vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second dose, sera and spleens were collected from all the animals for RT ELISA and IFN $\gamma$  ELISpot analyses, respectively. For all rodent immunizations, the Ad5 vectors were  
 5 diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadriceps muscles in 50  $\mu$ L aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following  
 10 vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second dose, sera and spleens were  
 15 collected from all the animals for RT ELISA and IFN $\gamma$  ELISpot analyses, respectively.

*Non-human Primate immunization* - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-lApol (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp dose; and (2) MRKAd5hCMV-lApol (E3-) at either  
 20 10<sup>9</sup> vp and 10<sup>11</sup> vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0)  
 25 into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

*Murine anti-RT and anti-nef ELISA* - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100  $\mu$ L of 1  $\mu$ g/mL HIV-1 RT protein  
 30 (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100  $\mu$ L of 1  $\mu$ g/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Huntsville, AL) and incubated for 2 h with 200  $\mu$ L/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was  
 35 performed followed by 4-fold serial dilution. 100- $\mu$ L aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100  $\mu$ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100  $\mu$ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100  $\mu$ L of 0.5M  $H_2SO_4$  per well. OD<sub>492</sub> readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD<sub>492</sub> (2.5 times the background value).

*Non-human primate and murine ELISpot assays* - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific IFN $\gamma$ -secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at  $5 \times 10^6$ /mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM  $\beta$ -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology*. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100  $\mu$ L/well of either 5  $\mu$ g/mL purified rat anti-mouse IFN- $\gamma$  IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15  $\mu$ g/mL mouse anti-human IFN- $\gamma$  IgG<sub>2a</sub> (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200  $\mu$ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50  $\mu$ L of cell samples ( $4 \times 10^5$  cells per well) and 50  $\mu$ L of the antigen solution were added. To the control well, 50  $\mu$ L of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4  $\mu$ g/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4<sup>+</sup>-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8<sup>+</sup>-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8<sup>+</sup> T cell epitope) or aa81-100 (CD4<sup>+</sup>) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO<sub>2</sub>, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 µL/well of either 1.25 µg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 µg/mL biotinylated anti-human IFN-γ goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 µL/well 1/2500 dilution of streptavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 µL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10<sup>6</sup> cell input.

*Non-human Primate anti-RT ELISA* - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 µL of each sample is incubated with 15 µL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN<sub>3</sub>) and 20 µL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 µL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

*Results - Rodent Studies* - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10<sup>7</sup> vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

Group	Vaccine	Dose	No. of Doses	Anti-RT IgG Titers <sup>a</sup>			SFC/10 <sup>6</sup> cells <sup>b</sup>		
				GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10 <sup>7</sup> vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(87) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10 <sup>9</sup> vp	2 1	1838400 <sup>b</sup> 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2083(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10 <sup>7</sup> vp	2 1	310419 6400	388218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2807(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10 <sup>9</sup> vp	2 1	1838400 <sup>b</sup> 1241675 <sup>b</sup>	0 396725	0 300681	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

<sup>a</sup>GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean

<sup>b</sup>Near or at the upper limit of the serial dilution; hence, could be greater than this value

<sup>c</sup>No. of Spot-forming Cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

- 5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this
- 10 model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELISpot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

Group	Vaccine	Dose	No. of Doses	Anti-nef IgG Titers <sup>a</sup>			SFC/10 <sup>6</sup> cells <sup>b</sup>		
				GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10 <sup>7</sup> vp	2 1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (E3+)	10 <sup>9</sup> vp	2 1	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnef (E3+)	10 <sup>7</sup> vp	2 1	132 115	42 46	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10 <sup>9</sup> vp	2 1	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10 <sup>7</sup> vp	2 1	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10 <sup>9</sup> vp	2 1	230 115	170 46	98 33	3(2) 7(1)	145(28) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	62	21(2)	18(6)	28(3)

<sup>a</sup>GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean

<sup>b</sup>No. of spot-forming cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

15

*Monkey Studies* - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

- peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of  $10^9$  vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10 Macaques.

Vaccine (T=0,4 wks)	Monkey #	Prebleed			T=4			T=7			T=16		
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IAPol(E3+) $10^{11}$ vp	99C100	1	0	0	1	38	31	0	52	146	0	49	715
	99C215	1	2	2	10	88	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IAPol(E3+) $10^9$ vp	99D212	0	2	0	4	331	114	0	58	14	0	6	6
	99D180	0	4	2	0	19	182	4	38	156	5	38	105
	99C201	8	5	21	6	62	62	0	18	32	1	14	65
MRKAd5hCMV-IAPol(E3-) $10^{11}$ vp	99D239	5	2	2	20	82	172	1	68	114	9	21	40
	99C186	4	12	6	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	464	0	14	238	1	24	284
MRKAd5hCMV-IAPol(E3-) $10^9$ vp	CC7C	10	10	8	12	724	745	4	322	378	4	188	178
	CD1G	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	6	6	12	10	88	110	5	60	80	8	25	34
Naïve	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined

Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN mMU/mL				
Vaccine/Monkey Tag	T=4	T=7	T=12	T=16
MRKAd5hCMV-IAPol(E3+), $10^{11}$ vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IAPol(E3+), $10^9$ vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IAPol(E3-), $10^{11}$ vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
99C084	235	2637	2858	1626
MRKAd5hCMV-IAPol(E3-), $10^9$ vp				
CC7C	32	175	306	235
CD1G	20	140	273	419
CD11	15	112	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Pre		T=4		T=7		T=16	
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 <sup>6</sup> 11 vp	CD2D	0	4	31	440	4	368	1	251
	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 <sup>6</sup> 9 vp	CC2K	9	9	6	52	0	35	0	15
	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 <sup>6</sup> 11 vp	99D191	1	5	4	614	0	298	2	419
	99D144	4	6	5	434	0	1100	2	932
	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 <sup>6</sup> 9 vp	99D224	1	11	14	231	1	125	0	70
	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naïve	083Q	nd	nd	18	22	4	5	2	1

#### EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B



gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapeutic advantage on a global scale.

5

Table 15  
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope # (from mapping)	mock	gag H-b	gagH-c	nef-b	nef-c
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

10

#### EXAMPLE 26

#### Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

15 *Expansion of nef and pol Adenovectors* - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

20

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 <sup>10</sup> vp/ml culture)	AEX Titer (10 <sup>4</sup> vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

- 5 *Roller Bottle Passaging* - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by
- 10 restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable ( $10^6$ cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) $10^{10}$ vp/ml culture	Titer $10^4$ vp/cell	Amplification Ratio	Triton Lysis Titer $10^{10}$ vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					
	2		1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable ( $10^6$ cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) $10^{10}$ vp/ml culture	Titer $10^4$ vp/cell	Amplification Ratio	Triton Lysis Titer $10^{10}$ vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%					
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

- MRKAd5nef and MRKAd5pol Viral Production Kinetics* - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6<sup>®</sup> cells in roller bottle cultures were infected at an MOI of 280
- 20 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral
- 25 particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

*Comparison of hCMV- and mCMV-FL-nef* - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6<sup>®</sup> cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10 <sup>6</sup> cells/ml), Viability (%)		Cell Passage Number	AEX Titer 10 <sup>10</sup> vp/ml culture	Titer 10 <sup>6</sup> vp/cell	Amplification Ratio	Triton Lysis Titer 10 <sup>10</sup> vp/ml culture
		Infection	Harvest					
hCMV-FL-nef (MRKAd5nef)	Pool	1.11, 91%		60	1.5	1.4	50	2.8
	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

20

### EXAMPLE 27

#### Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

*Materials and Methods* - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6<sup>®</sup> cells at a concentration of 0.2x10<sup>6</sup> cells/ml. Cells were grown until they reached a cell concentration of approximately 1x10<sup>6</sup> cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

- 5 were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

10

Run	Batch ID	Cloned/Unclosed MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Unclosed	280
	B20010115-2	Unclosed	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

*Results* - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

15

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Unclosed MRKAd5nef	Virus Concentration @ 48hpi (1x10 <sup>13</sup> vp/L)			
			Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Unclosed	0.72	3.26	3.98	5.76
	B20010115-2	Unclosed	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Unclosed MRKAd5nef	Virus Concentration @ 48hpi (1x10 <sup>11</sup> IU/L)				
			Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Unclosed	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Unclosed	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

20

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

#### EXAMPLE 28

##### 5 MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pV1JnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of  
10 V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of  $10^7$  viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note:  $10^7$  is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

15 Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50  
20 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

25 The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced,  $CD4^+$ -biased or  $CD8^+$ -biased, and (b) boosting with the MRKAd5gag  
30 construct produced in all cases a strongly  $CD8^+$ -biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific  $CD8^+$  T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKA45g99

Group	Priming T=0, 4, 8 wks DNA/5 mgs PBS (0101)	Boost T=28 wks MRKA45g99(E3+) 10 <sup>-7</sup> vp	Monkey	T=0		T=4		T=6		T=10		T=17		T=24		T=28		T=30	
				Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H
1	DNA/5 mgs + CRL1005/45 mgs	MRKA45g99(E3+) 10 <sup>-7</sup> vp	CB5H	NA	NA	3	35	15	71	4	224	8	119	0	85	19	858	0	316
			CB5K	0	0	0	15	0	46	0	68	0	76	0	35	3	1705	1	755
			AN2G	5	11	0	36	3	51	3	46	2	89	8	65	10	889	0	395
2	DNA/5 mgs + CRL1005/45 mgs	MRKA45g99(E3+) 10 <sup>-7</sup> vp	CC1C	0	4	1	60	0	111	5	270	4	280	8	232	3	959	19	1345
			CC1K	4	0	1	101	0	284	0	781	5	482	0	321	0	1915	1	1069
			AW3P	9	8	1	10	4	71	4	154	6	104	6	85	11	838	6	241
	DNA/5 mgs + CRL1005/7.5 mgs + 0.5 mM BAK	MRKA45g99(E3+) 10 <sup>-7</sup> vp	CB5F	NA	NA	0	31	0	288	0	530	19	374	0	251	8	1549	20	1734
			AK8B	9	12	4	36	1	119	0	439	0	425	0	316	4	1229	5	1354
			AW2D	10	4	1	59	5	264	19	425	8	105	9	205	18	565	9	404
3	DNA/5 mgs + CRL1005/7.5 mgs + 0.5 mM BAK	MRKA45g99(E3+) 10 <sup>-7</sup> vp	CA4R	1	0	3	121	1	135	1	270	5	130	1	105	14	1384	10	978
			CB5B	8	6	0	6	3	119	0	274	6	282	1	208	0	608	1	828
			CB5W	4	3	0	28	1	81	0	139	0	184	1	82	5	643	1	349
	none	None	CB7D	1	0	0	136	0	316	1	609	5	628	1	769	0	2278	4	1631
4			96D201	3	0	0	0	1	0	0	0	0	1	1	2	3	0	0	0

NA, not available

## EXAMPLE 29

## Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNaseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNase H and integrase (1350 amino acids; SEQ ID NO: 39).

The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IAPol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IAPol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IAPol fusion gene.

## EXAMPLE 30

## Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with  $10^8$  or  $10^{10}$  viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN- $\gamma$  ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

**Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques**

Grp #	Vaccine T=0, 4 wks	Monk #	T=6 wks				
			Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag 10 <sup>10</sup> vp	CB9V	0	15	-	-	-
		CD19	0	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag 10 <sup>8</sup> vp	99D130	1	948	-	-	-
		W277	16	324	-	-	-
		143H	4	595	-	-	-
3	MRKAd5 pol 10 <sup>10</sup> vp	CC1X	4	-	46	256	-
		AW3W	3	-	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol 10 <sup>8</sup> vp	AW38	1	-	19	30	-
		CC8K	0	-	50	995	-
		CC21	1	-	33	436	-
5	MRKAd5 nef 10 <sup>10</sup> vp	076Q	9	-	-	-	1204
		091Q	4	-	-	-	85
		083Q	0	-	-	-	176
6	MRKAd5 nef 10 <sup>8</sup> vp	00C029	1	-	-	-	114
		98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 <sup>10</sup> vp each	99D251	3	206	15	193	120
		05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 <sup>8</sup> vp each	99D215	1	171	18	193	240
		81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef 10 <sup>10</sup> vp each	99D211	0	83	56	838	725
		22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef 10 <sup>8</sup> vp each	34H	3	78	19	5	75
		48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCs against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10<sup>6</sup> PBMC.



## WHAT IS CLAIMED IS

:

1. A recombinant adenoviral vaccine vector at least partially deleted in  
5 E1 and devoid of E1 activity, comprising:
  - a) an adenovirus *cis*-acting packaging region corresponding to from  
about base pair 1 to between from about base pair 400 to about  
base pair 458 of a wildtype adenovirus genome; and
  - b) a gene encoding an HIV protein or immunologically relevant  
10 modification thereof.
2. A vector in accordance with claim 1 comprising a packaging region  
corresponding to from about base pair 1 to about base pair 450 of a wildtype  
adenovirus genome.
3. A vector in accordance with claim 1 further comprising nucleotides  
15 corresponding to between from about base pair 3511 to about 3524 to about base pair  
5798 of a wildtype adenovirus genome.
4. A vector in accordance with claim 3 comprising base pairs  
corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
5. A vector in accordance with claim 4 which is deleted of base pairs  
20 451-3510.
6. A vector in accordance with claim 1 which is at least partially  
deleted in E3.
7. A vector in accordance with claim 6 wherein the E3 deleted region  
is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a  
5 gene expression cassette comprising:

- a) a nucleic acid encoding a protein;
- b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
- (c) a transcription termination sequence.

10 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene  
15 expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.

20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

18. A cell comprising the adenoviral vector of claim 1.

19. Recombinant, replication-defective adenovirus particles harvested  
5 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.

20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.

21. An HIV vaccine composition of claim 20 which comprises a  
10 physiologically acceptable carrier.

22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant,  
15 replication-defective adenovirus.

23. A method according to claim 22 wherein the cell is a PER.C6<sup>®</sup> cell.

24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of  
20 claim 21.

25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) SEQ ID NO: 29;
  - ii) a heterologous promoter operatively linked to i); and
  - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

5           34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

10           36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell  
15   line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

20           41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6<sup>®</sup> cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of  
5 claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

10 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

15 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

20 49. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- 5 a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
  - ii) a heterologous promoter operatively linked to i); and
  - 10 iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

15 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

20 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus  
5 particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of  
10 claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6<sup>®</sup> cell.

62. A method of generating a cellular-mediated immune response  
15 against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with  
20 a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.



65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

5           67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

10           69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs  
15           corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising  
i) a nucleotide sequence selected the group consisting of  
SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and  
20           SEQ ID NO: 15;  
ii) a heterologous promoter operatively linked to i); and  
iii) a transcription termination sequence.

70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

10 75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

15 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

20

80. A method according to claim 79 wherein the cell is a PER.C6<sup>®</sup> cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises  
5 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus  
10 vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

15 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a  
20 gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

- a) gag, pol, and nef, expressed independently from three individual vectors;

- 5           b) gag, pol, and nef, expressed independently from one vector with  
the encoding nucleic acid sequences operatively linked to distinct  
promoters and transcription termination sequences;
- c) gag, pol, and nef, expressed via two vectors, one expressing a pol-  
nef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gag-  
pol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nef-  
gag fusion and another expressing pol;
- 10          f) gag, pol, and nef, expressed via one vector expressing a gag-pol-  
nef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the  
encoding nucleic acid sequences operatively linked to distinct  
promoters and transcription termination sequences;
- 15          i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the  
encoding nucleic acid sequences operatively linked to distinct  
promoters and transcription termination sequences;
- 20          k) nef and gag, expressed independently from two individual vectors;
- l) nef and gag, expressed independently from one vector with the  
encoding nucleic acid sequences operatively linked to distinct  
promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion;

and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with  
5 claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with  
claim 86 wherein the fused sequences have the encoding nucleic acid sequences  
operatively linked to distinct promoters and transcription termination sequences.

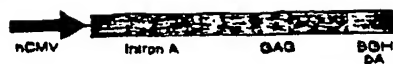
89. A multivalent adenovirus vaccine composition in accordance with  
10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences  
operatively linked to a single promoter; and the encoding nucleic acid sequences  
operatively linked by an internal ribosome entry sequence ("IRES").



[illegible]

2/144

Old Transgene:



New Transgenes:

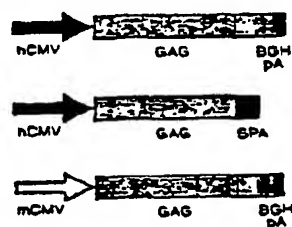
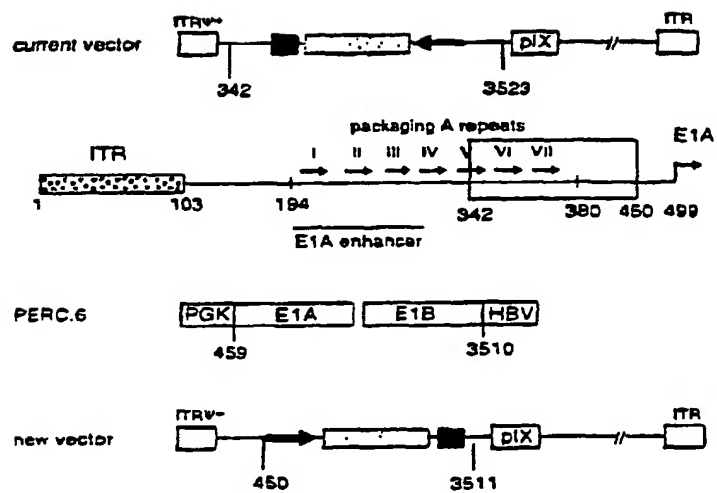
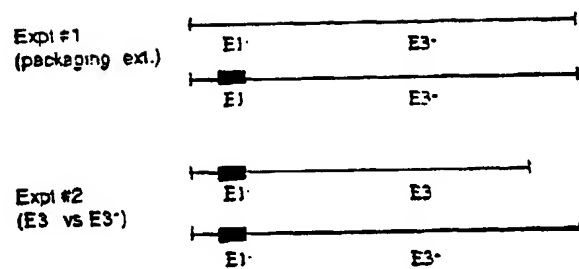


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

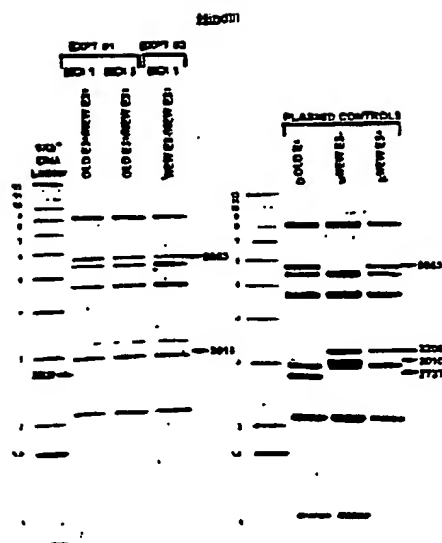




**Figure 4:** Modifications made to the current adenovector backbone in the generation of the new vector.



**Figure 5:** Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.



**Figure 6:** Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

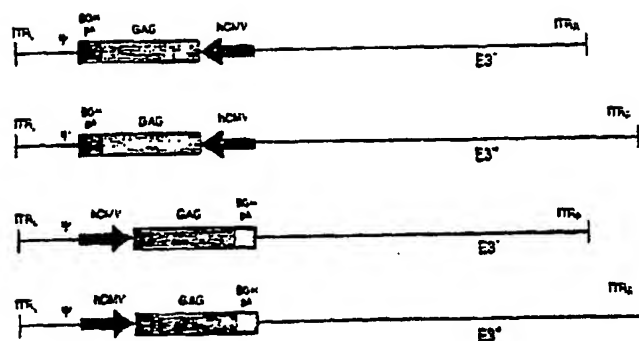
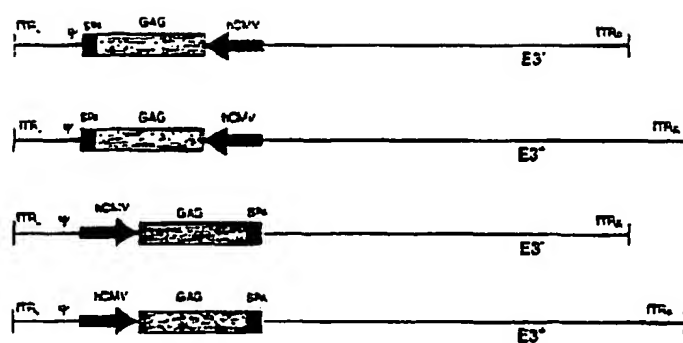
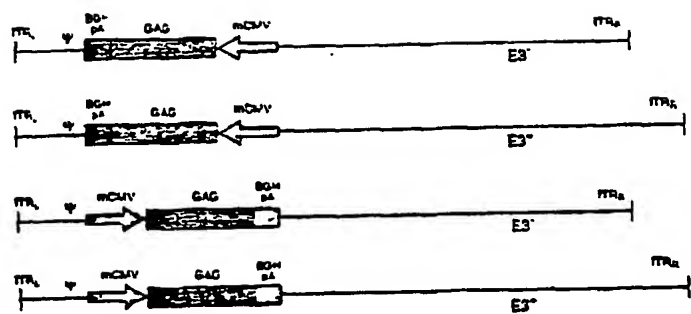


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.



**Figure 7B:** hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.



**Figure 7C:** mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

## Plasmid mixing expt: (orientation)

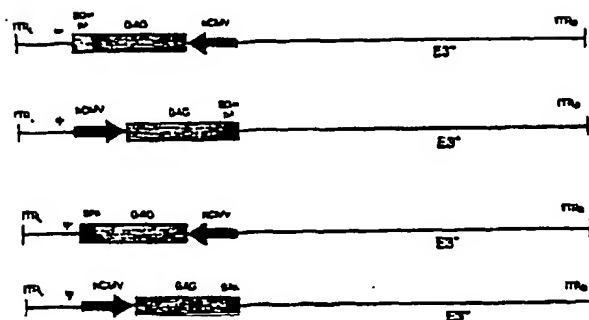
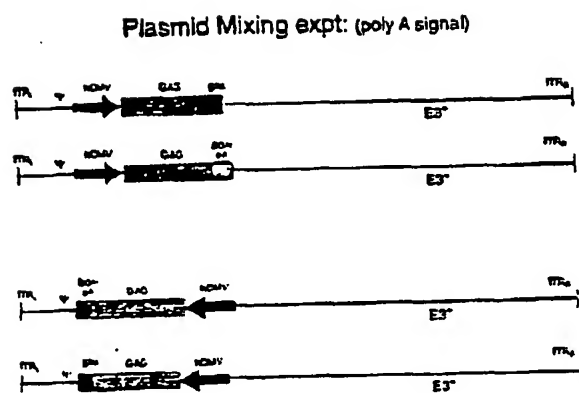


Figure 8A: Effect of transgene orientation



**Figure 8B: Effect of polyadenylation signal**



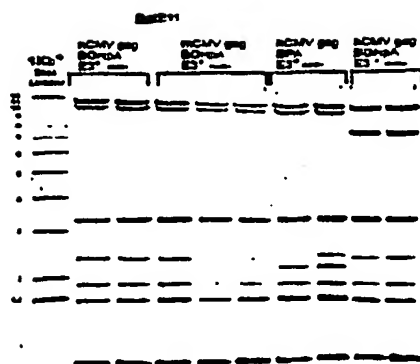


Figure 9: Viral DNA from the four Adgag candidates at P5, following BstE11 digestion.

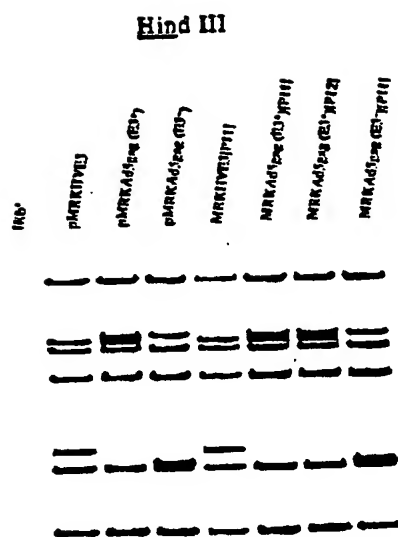
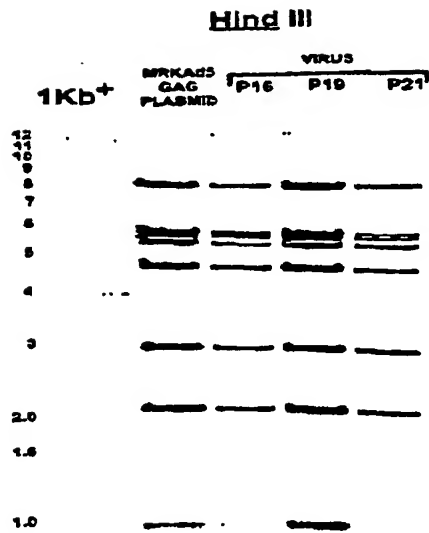


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

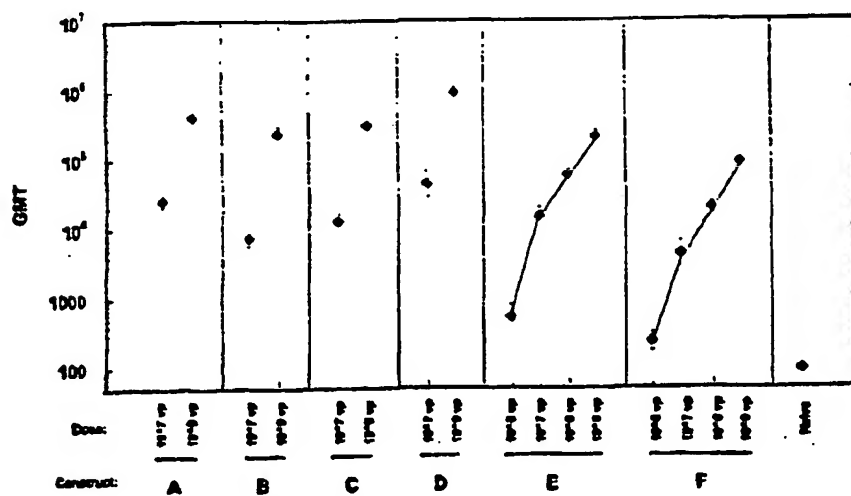


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).



**Figure 12:** Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21 (serum containing media).

13  
 Figure 1. Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3<sup>-</sup> bCMV-FLgag-bGHpA; (C) MRKAd5 E3<sup>-</sup> bCMV-FLgag-SPA; (D) MRKAd5 E3<sup>-</sup> mCMV-FLgag-bGHpA; (E) research lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



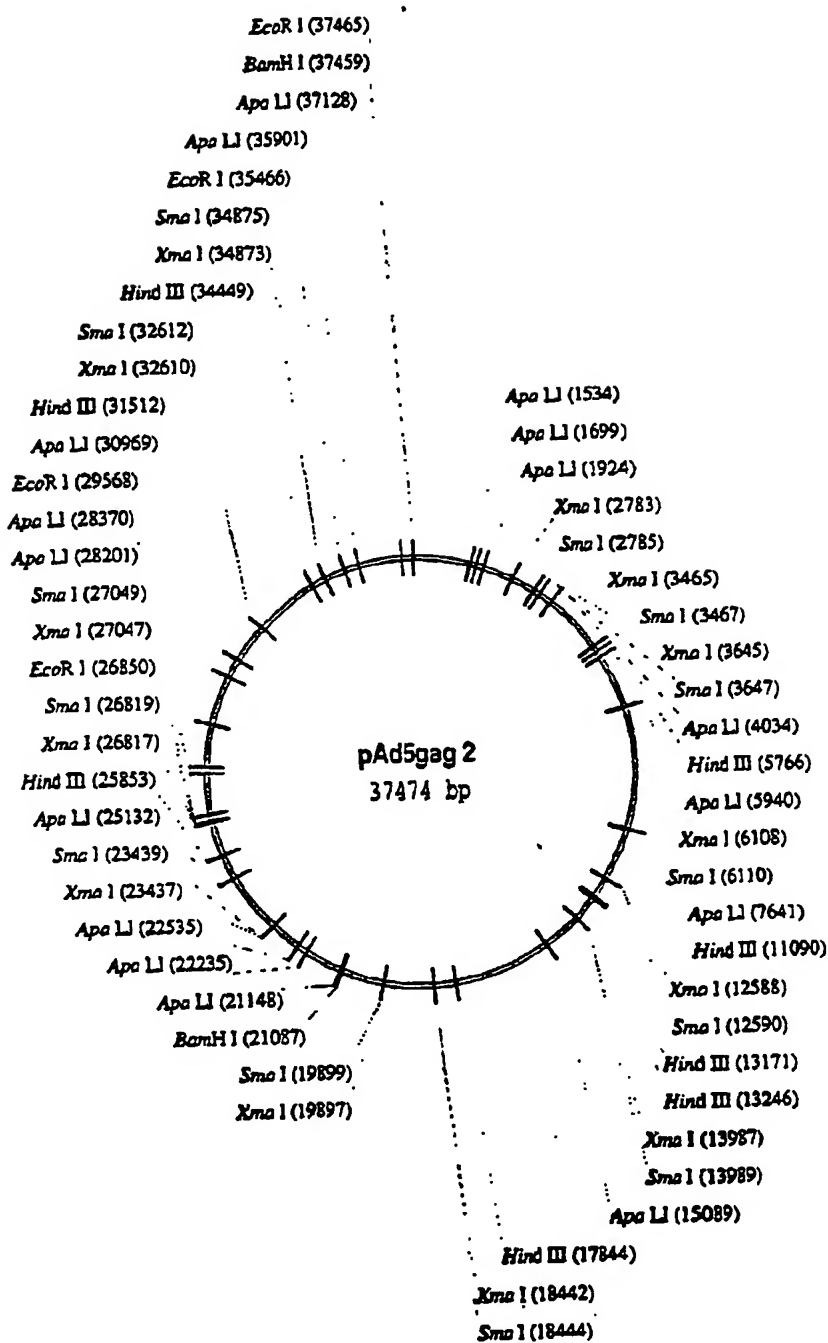


Figure 14

PARKAV59991 MER682

1	TTCTTAATTA ACATATCAAA TAATATATCT TATTTTGTAT TTATATATAT ATGATATATTA GGTATATATAG TTTTATATCTT GGTATATATCTT GGTATATATCTT GGTATATATCTT
101	AGATATATAT TATATATATCT ATATATATCTA ATATATATCTA ATATATATCTA ATATATATCTA ATATATATCTA ATATATATCTA ATATATATCTA ATATATATCTA ATATATATCTA
201	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
301	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
401	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
501	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
601	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
701	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
801	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
901	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
1001	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
1101	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
1201	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
1301	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
1401	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
1501	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA
1601	GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA GTATATATCTA

Figure 15A

## pNRKAF19191 HER682

1701 CACCAAGGCA TCTCCGCGCG GACCTGATAT GGCATGATGTA AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 GTGCTCCGCT AGAGCGGATC CTGTGATCTTA GCGAGCCACT TTGAGCATCT CTCTCTCCCT GATAGGAGAC TCCACTAGCG GTACAGAGAG CCGTTCAGAC  
 1801 AGGTGCGAC CCGCCAGGAC CTGTGATCTTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 TCCAGCGTG GCGGTGCTG GACTGATGAT ACTGATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 1901 TGAATGCGAC AGGTGCGAC CTGTGATCTTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 ACTGATGCG TCCGATGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2001 CAGAGCGCA TTGCTGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 GTGCTGCTCT ACCGAGCTTA CTGTGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2101 ACTGATGCG TCCGATGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 TGTGATGCG TCCGATGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2201 CAGAGCGCA TTGCTGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 GTGCTGCTCT ACCGAGCTTA CTGTGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2301 GAGAGCGCA TTGCTGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 GTGCTGCTCT ACCGAGCTTA CTGTGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2401 TGAATGCGAC AGGTGCGAC CTGTGATCTTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 ACTGATGCG TCCGATGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2501 GAGAGCGCA TTGCTGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 GTGCTGCTCT ACCGAGCTTA CTGTGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2601 AGGTGCGAC CCGCCAGGAC CTGTGATCTTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 TCCGATGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2701 AGGTGCGAC CCGCCAGGAC CTGTGATCTTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 GTGCTGCTCT ACCGAGCTTA CTGTGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2801 CAGAGCGCA TTGCTGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 GTGCTGCTCT ACCGAGCTTA CTGTGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 2901 TGAATGCGAC AGGTGCGAC CTGTGATCTTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 ACTGATGCG TCCGATGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 3001 GAGAGCGCA TTGCTGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 GTGCTGCTCT ACCGAGCTTA CTGTGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 3101 TGAATGCGAC AGGTGCGAC CTGTGATCTTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 ACTGATGCG TCCGATGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 3201 GAGAGCGCA TTGCTGATG GACATGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG  
 GTGCTGCTCT ACCGAGCTTA CTGTGATGTA TGTATGATAC AATGATGATGTA GAGAGAGGTC TTCTCCCTG AGGTGATCCC CATGTTCTCT GCGTGTGCTG

Figure 15B



[illegible]

Figure 15c

[illegible]

Figure 150

[illegible]



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9701	ACAAAGGCGGT GGTATGCGCC CGTTTGTGATG TTGTATGATG AGTGTGCGAT ACTGATGAG TTATGCGTCT GGTATGCGCG CTGCGAGAGC TCGTGTATATC TCGTGTATATC
	TOPTTGGGCA CCATAGCGCG GCACAACTAC CAAATGACG TCAACCTGAT TTTCTGTGTC AATTGCGAGA CCACTGTGCG GACGCTCTCG GACGCTCTCG AGCCGATATC
9801	TGAGAGCGCA GTAAAGCGTC GAGTCAATA CTATATGCTT GTATGCTTTC ATCTGTACT GTATGCGAC CAATAATGCG GCGCGGCGGT GCGGTGTAGAT GCGGTGTAGAT
	ACTCTGCGCT CAFTGCGAG CTCAGTTTAT GCATCAATTA GGTTCAGAGG TATATGATTA CCATAGCGCG GTTTTTCAGC CCGCGCGCGA CCGGTATCTC
9901	GCGCGAGCGT AGGTGTGCGG GCGCTGCGCG GCGCGAGCT GTATGATTA GCGCGATTA TCTGTGATG TACTGTGACA TCCAGGTGAT TCCAGGTGAT GCGCGCGCGC
	CGCGGTGCGA TCCAGCGCGC CCGCGAGCGC CCGCTCTAGA AGGTGTGAT CCGCTACTAT AGCTATCTAG ATGAGAGCTGT AGGTGAGCTA TCCAGGTGAT GCGCGCGCGC
10001	GTGTGTGAGG CGCGCGGAA GTGTGTGAGG CGGTGTGAG GTGTGTGAG GTGTGTGAG GTGTGTGAG GTGTGTGAG GTGTGTGAG GTGTGTGAG GTGTGTGAG
	CAGCGAGCGC GCGCGCGCTT CAGCGCGCTT CAGCGCGCTT CAGCGCGCTT CAGCGCGCTT CAGCGCGCTT CAGCGCGCTT CAGCGCGCTT CAGCGCGCTT CAGCGCGCTT
10101	AATGTTTAC GCTCTAGCG GTGCAAGCG GTGCAAGCG GTGCAAGCG GTGCAAGCG GTGCAAGCG GTGCAAGCG GTGCAAGCG GTGCAAGCG GTGCAAGCG
	TTAGCAACTG CGAGATCTCG CAGCTTTTC TCTGCGCAT TCGCGCGCTA GAGGAGCTA GAGGAGCTA GAGGAGCTA GAGGAGCTA GAGGAGCTA GAGGAGCTA
10201	GCGTGTGAGC CCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
10301	TTGTGCTTC TTGCGCGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	AAAGCGGAGG AAGTGTGCG CCGCGCGCG CCGCGCGCG CCGCGCGCG CCGCGCGCG CCGCGCGCG CCGCGCGCG CCGCGCGCG CCGCGCGCG CCGCGCGCG
10401	GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
10501	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
10601	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
10701	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
10801	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
10901	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
11001	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
11101	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
11201	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG
	CGCGAGCTCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG GCGGTATGCG

Figure 15g

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11301	TCGATTTGAT AACATCTCG CAGGATATG TGTCTTACA GTTAAATTG AGTTATATC ATATGTTGAT CCGCATAC TATTCATGC TTAGCTGTT
11401	AGCTAACTA TTCTTAGGAC GTCTCTATC ACATCTTTC TCTTCTTATC TTCTATGACG TTTTCTACGC TTTTCTACGC TATTCATGC TATTCATGC
11501	CAGGTTTAC GCGCGAAGA TATATCTAC CATTACTCT CATTATACA ATATATATG TATCTATGAT TATCTATGAT TATCTATGAT TATCTATGAT
11601	GTTCMAATG CCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
11701	ACCTTATCG ACCATCTCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
11801	TCGATCTGC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
11901	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
12001	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
12101	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
12201	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
12301	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
12401	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
12501	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
12601	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
12701	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG
12801	CGATCTGTC GCGGCTTCT ATATGATG GCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG CCGAATGCG

Figure 15H

## DHKAd'ing MFR6R2

12901 GAGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13001 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13101 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13201 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13301 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13401 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13501 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13601 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13701 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13801 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 13901 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 14001 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 14101 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 14201 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 14301 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG  
 14401 GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG GGTGATGATG

Figure 151



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14501	CGTACGATTA TCTGAGGCT GTTACATTC CGGACGCTT GTATATGAC GTTACATTA AGATGACAGC GAAAGAGGCG GGGGTGCTTC
14601	GGATCTACT AGAGCTGCTA CGATTTTAA GGCCTACAAA GCTACATTTG CGTACGACTT TCTACGATTC CTCTCCCGC CCGACCGCTT
14701	AGGTGGGAGC AACAGAGCTG GGTATGAGC GTTACGAGC GTTACGAGC GTTACGAGC GTTACGAGC GTTACGAGC GTTACGAGC
14801	AGAGAGACC GGTATGAAA GGCCTGACAG AGTACATTA GAAAGCTAT TACAGCTAA TACAGCTAA GAGGACCTTC ACCGATTAAC GAGGTGCTTA
14901	CTCTGATAC ACTAGAGCG ACCCTGACAG CGTATATTC TCTATGATC TCTATGATC TCTATGATC TCTATGATC TCTATGATC
15001	GTGAGGATTA GTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
15101	GTCTGATTA GAGGAGAGCG GTCTGATTC ACTATATCG GATGATATCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
15201	CGGAGGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
15301	GTGAGGATTA GTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
15401	GTATGATTA GTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
15501	GTATGATTA GTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
15601	GTATGATTA GTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
15701	GTATGATTA GTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
15801	GTATGATTA GTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
15901	GTATGATTA GTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG
16001	GTATGATTA GTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG GTTATGAGCG

Figure 15J



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16101	CCAGTTCATC GCGCCGAGCA TCTATGCTC CCGTANGAGG GAGGAGAGAG ATTATAGCTC CTGAGAGCTA AGCGGCTCA AAGAGAGAAA GAAAGATGAT	CGTCCAGTAG CCGGCGCTCT AGATACCGGCG GCGCTCTTTC CTT TCTGTC TAAATCTCTG) GCTCTTGCAAT TTGCGCGAAT TTCTCTTTT CTTTCTACT
16201	GATGATGAGC TTGACGACGA GGTGAGATTC GTTGTTCAG: TGTATATGTTA GATGAGAGG GTGAGAGGCTT AAGAGCTGTT TTGGGATCC	CTACTACTTG AACTGCTGCT CCACTCTGAC GAGCTGAGAT GGTATGCTTC: TCTCTGCTTC GTACCTGCGCA TTTCGACAAA AGCGCTGCTG
16301	GCACGACGCT AGTCTTTTACG CCGCTTGAGC GCTCCAGCGC GACTATACAG GCTGTGTATG ATGAGGTTTA GCGGCGATAG GAGCTGCTTA AGCAGGCTCA	CGTGTGTGCA TCGAGAGATGC TCGGCGCTGCG: GTGATGTTTC GTATGAGTAC TACTGCTGCTC CTGAGAGAAC TCGTCTGCTTT
16401	CGAGGCTGTC GGTGAGTTTG CCTACGAGAA TCGGATGAG GAGATGTTTG: TCTTGTCTCT GTACGAGTGC AGCCGAGTAC CTAGGCTAAA GCGGCTTAA	GCTGCGGAG GCGCTCAAG CCAATGCTTT CCGCTGATTC CTGTAGAGC GCAATGAGTA CCGTCTGCGG TTGGTGTGTC GATCGGATTT CCGGCTATGT
16501	CTGAGGAGAG TCGTCCGCGC GCTTGTACG TCGGAGAGAA AGTCTGCTCT: AAGGTAGAG TTCTGCTTACT TTGACGCGAC GGTGAGGCTG ATGCTTATGCA	GAGCTGCTTC AGCTGCTGCG CCACTGCTGC AGCTTCTTT TCGGCTGCTA TTCTGCTGTC AGAGCTGCTA AGCTGCTGAC TACCATGCTT
16601	AGCGGAGCGC ACTGAGAGAT GTCTTGAGAA AATGAGCTGC GTAGCTGCTG: GTAGCTGCTG AGCTGCTGCT GCGGCTGCTG CCGGCTGCTG	TGCGGCTGCG TGAAGCTTCA GAGAGCTTTT TTACTGCTGA CTTTGTAGTC GAGCTGCTGCT TCGGCTGCTG CCGGCTGCTG CCGGCTGCTG
16701	GCGGCTGCTG AGCTGCTGCG TTGAGATGC CACTAGCTCT AGAGCTGTTA TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG	CGCGGCTGCG TCGGCTGCTG AGCTGCTGCG GTAGCTGCTG TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG CCGGCTGCTG
16801	GCGGCTGCTG AGCTGCTGCG GTAGCTGCTG CACTAGCTCT AGAGCTGTTA TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG	CGCGGCTGCG TCGGCTGCTG AGCTGCTGCG GTAGCTGCTG TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG CCGGCTGCTG
16901	GCGGCTGCTG AGCTGCTGCG GTAGCTGCTG CACTAGCTCT AGAGCTGTTA TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG	CGCGGCTGCG TCGGCTGCTG AGCTGCTGCG GTAGCTGCTG TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG CCGGCTGCTG
17001	GAGCTAGCGC CCGAGAGAGC GAGGAGTATC CCGAGCGCGA AGCTGCTGCT: GAGGAGTATC CCGAGCGCGA AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG	GTGAGTATGCG GGTGCTTCTG CTGCTTGTATG GGTGCTGCTG TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG CCGGCTGCTG
17101	GTGAGTATGCG GGTGCTTCTG CTGCTTGTATG GGTGCTGCTG TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG CCGGCTGCTG	GTGAGTATGCG GGTGCTTCTG CTGCTTGTATG GGTGCTGCTG TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG CCGGCTGCTG CCGGCTGCTG
17201	ATATGCTGCT GAGCTGCTGC CCGCTGCTGC GTGCTGCTGC ATGCTGCTGC: ATGCTGCTGC GTGCTGCTGC CAGGCTGCTGC CCGGCTGCTGC	TATAGCTGCTA GTGAGTATGCG GAGGAGAGCG GCGAGCGCGC TAAAGCTGCT: TCTTATGCTG CAGTCTGCTG GTAGGCTGCT GCGGCTGCTG
17301	CGCTGCTGCG CAGGAGCGCG GCGGCTGCTG GTAGGAGCTG CTATGCTGCT: GCGGCTGCTG GCGGCTGCTG ATGCTGCTGC TCGGCTGCTG	CGGAGAGCGC GTGCTGCTGCG CCGGCTGCTG GAGGAGTATG CCGGCTGCTG: GCGGCTGCTG GCGGCTGCTG ATGCTGCTGC TCGGCTGCTG
17401	GTGCTGCTGC TCGGCTGCTG GCGGCTGCTG GCGGAGTATC AGCTGCTGCT: TCGGCTGCTG GCGGCTGCTG ATGCTGCTGC TCGGCTGCTG	CAGGCTGCTG AGCTGCTGCG CCGGCTGCTG TCGGCTGCTG TCGGCTGCTG: TCGGCTGCTG TCGGCTGCTG ATGCTGCTGC TCGGCTGCTG
17501	GCGGCTGCTG TCGGCTGCTG TCGGCTGCTG GAGGAGTATC AGCTGCTGCT: TCGGCTGCTG TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG	GCGGCTGCTG AGCTGCTGCG CCGGCTGCTG TCGGCTGCTG GAGGAGTATC: TCGGCTGCTG TCGGCTGCTG AGCTGCTGCT TCGGCTGCTG

Figure 15K

Year	Event	Location	Notes
1760	First recorded earthquake	San Francisco	Estimated magnitude 4.5
1770	Major earthquake	San Francisco	Magnitude 5.5
1780	Minor earthquake	San Francisco	Magnitude 3.5
1790	Major earthquake	San Francisco	Magnitude 5.0
1800	Minor earthquake	San Francisco	Magnitude 3.0
1810	Major earthquake	San Francisco	Magnitude 5.0
1820	Minor earthquake	San Francisco	Magnitude 3.5
1830	Major earthquake	San Francisco	Magnitude 5.0
1840	Minor earthquake	San Francisco	Magnitude 3.5
1850	Major earthquake	San Francisco	Magnitude 5.0
1860	Minor earthquake	San Francisco	Magnitude 3.5
1870	Major earthquake	San Francisco	Magnitude 5.0
1880	Minor earthquake	San Francisco	Magnitude 3.5
1890	Major earthquake	San Francisco	Magnitude 5.0
1900	Minor earthquake	San Francisco	Magnitude 3.5
1910	Major earthquake	San Francisco	Magnitude 5.0
1920	Minor earthquake	San Francisco	Magnitude 3.5

Figure 15L

pHRKAD59a9 MER682

19301 AGACATTAAG GGCACACAT CTATCTCA CAGCTCTAT TACATCTCT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 TCTTGATTAAC CCGATCTGTA GATACCTAT GTCTCTATTA ATTTATACGA ATATCTCTTT AATATATACA GATTACATTA GATTATATTA TTTTATATTA  
 19401 GGTGTCTTGG GGGGCGAAGC ATCTATATTA ATATCTCTTT TACATCTCT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 CCACAGAGCC GCGGCTCTCG TACGCTCAAC TTATATATTA ATATCTCTTT TACATCTCT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 19501 ATAGACACAG GTACTCTTCT ATATCTCTTT TACATCTCT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 TATCTCTCTC CATGAAAGGA TACATCTCT TTATATATTA ATATCTCTTT TACATCTCT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 19601 TTACTCTCTT CAGCTCTGAG GTCTCTATTA TACATCTCT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 AATGACUAA GGTGACCTCT CACATCTAT ATCTCTCTTA GATCTCTCT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 19701 TTCTCTGATA AATATGAAAT AAGATCTGTA ATATCTCTTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 AATATCTCTT TTCTCTATTA TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 19801 TGTATTTGTC GACACAGCTA AATCTCTCT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 ACATTAAGCG GCTCTCTGAT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 19901 GCTAGTCTAC TGTCTCATTA AATCTCTCT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 GATCTCTCTT AATATCTCTT TGTCTCATTA AATCTCTCT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20001 GCTCTCAATG TGTCTCTGTA TGTCTCTCT GATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 GGTAGTTACA AGGACCTCTT ACCAGCTGTA CAGCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20101 ACACCTCTCA GTGACCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 TGTCTCTCTT CAGCTCTGTA TGTCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20201 CATCTCTCTT TACCTCTCTT TGTCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 GTAAACCTGA ATCTCTCTCT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20301 TATCTCTCTT CAGCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 ATAGACCTCT GGTCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20401 CCTCTCTCTT GGTCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 GATCTCTCTT GGTCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20501 TTACTCTCTT CACACTCTTA AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 AATCTCTCTT GGTCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20601 AAGCTCTCTT TGTCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 TTCTCTCTT TGTCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20701 AGCTCTCTT TATCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 TGTCTCTCTT TGTCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20801 GATCTCTCTT CAGCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 CCTCTCTCTT GGTCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 20901 TGTCTCTCTT CAGCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT  
 AAGCTCTCTT GGTCTCTCTT AATCTCTCT TTCTCTATTT TTCTCTATTT TTATATATTA TTATATATTA CTATATATTA ACACAGCAG CCGTAAATAT

Figure 15M

pMRK45-9-99 MEIR612

21001	TTATGTCGAT GGGGCGACT ACAGACCTG ACCAAGCTT TCTTATATTC ACTTCCCTCC ACAGCGTACA CATGACTTTT GAGTGTGATC CATTGTACBA CATTGTACBA	Band II
21101	ATATACAGCTA CCGCGCTGAG TGTCTGACCC GATTTTGTGA ATGATATATTA TTGATATATTA TGTGATATTA TGTGATATTA TGTGATATTA TGTGATATTA TGTGATATTA	Fig II
21201	TCGCGCGGCA AGCGGCAAC ATAGAGAGC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC	Fig III
21301	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig IV
21401	ATAGAGAGC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC AGCGAGATC	Fig V
21501	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig VI
21601	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig VII
21701	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig VIII
21801	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig IX
21901	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig X
22001	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig XI
22101	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig XII
22201	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig XIII
22301	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig XIV
22401	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig XV
22501	TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT TCGCGCGGCT	Fig XVI

Figure 15N

PHRKA[5]gany MER682

22601 ATCTTGGCTT TACTAGACTG CTCTCTTACG GCGGCTCTTC CTTTCTTCTT ATTCTAATTA CTCTCTCTCTT ATTCTAATTA ATCTTCTCTT ATCTTCTCTT  
TAGAACCGGA AGGATCTGAC GATCTGATCT GCGGCTCTTC CTTTCTTCTT TAACTTCTT GACGAGGGA TAACTTCTT TACGAGGGA  
Pall

22701 GTAGACACTT AAGCTGGCTT TCGATTCTG TCTAGCTGCT CACTCTACAC CTCTCTCTCTT TCGCTCTCTT ATCTTCTCTT GTCACCTCTT CAACTCTCTT  
CATCTCTGAA TCTGAGCGGA AGCTAGACTG GCGGCTCTTC CTTTCTTCTT TAACTTCTT ACCGCTCTT TACGAGGGA CACTCTCTT  
Pall

22801 CAGCTACGCT TCGAGGACT GCGGCTCTTC CACTCTACAC CTCTCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
GCTCTCTCTT TCGAGGACT GCGGCTCTTC CACTCTACAC CTCTCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

22901 CATACGCTCT CACTCTCTTC CACTCTCTTC TAACTTCTT TAACTTCTT TAACTTCTT TAACTTCTT TAACTTCTT TAACTTCTT  
GTATCTCTCT GCTCTCTCTT GCTCTCTCTT CACTCTCTTC CACTCTCTTC TAACTTCTT TAACTTCTT TAACTTCTT TAACTTCTT  
Pall

23001 GATCTCTCTT CTCTCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
GATCTCTCTT GATCTCTCTT CTCTCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

23101 TCGCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
GATCTCTCTT GATCTCTCTT CTCTCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

23201 ACCATCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

23301 TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
GATCTCTCTT GATCTCTCTT CTCTCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

23401 TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
GATCTCTCTT GATCTCTCTT CTCTCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

23501 GATCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

23601 GATCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

23701 GATCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

23801 GATCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

23901 GATCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

24001 GATCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

24101 GATCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
TCTCTCTCTT GATCTCTTC GATCTCTTC GCGGCTCTTC CACTCTCTTC TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT TCGCTCTCTT  
Pall

Figure 150

## pMRKad5gag MER6R2

24201	CTGTCGCTGAA CCGAGTCTTTC AGATCTTTTC AGCTGATGAG AAGTGTGTTC CAAGATCTCT GTGACAGGGA AACAGCTGAA ATGATAGTCA GAGGCGATTT GTTTAGAAAC TCCGAGAAC TATATCTCTC TTCTGTCTCC GTTTGCTGTA GTTTGTCTTT TTGTGCTTTT TACTTTTCAAT
24301	CTCTGGAGTG TTGTGTGAAAC TGTAGGTGTA GAGCTGTCTT CTATCTCTAC TAAATCTGAG CATCTGATTC ACCCTCTTTC GTTACCTGAC ACTTTAACCTTA
24401	GAGACCTTAC AACCACTTTC AGCTCCCACT GTTTGCTGAG GATCTGATTC ATTTTGTGTC GTATCTGAG TATCTGTGAG GATATGAGCTT TGAATTTGAT
24501	CCCCCAAGG TCAATGAGAC AGTCAATGAGT GATCTGATTC TATCTGTGAG TATCTGTGAG TATCTGTGAG TATCTGTGAG TATCTGTGAG TATCTGTGAG
24601	TACCGGCAAT TCGCGAGGAG CAGCTAGTTC AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA
24701	TACCGGCAAT TCGCGAGGAG CAGCTAGTTC AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA AGCTGCTTCA
24801	CTCTCAAGGG GAGGCGGCG GCGCTGATTC AGCGCTGATTC AGCGCTGATTC AGCGCTGATTC AGCGCTGATTC AGCGCTGATTC AGCGCTGATTC AGCGCTGATTC
24901	GAGGAGGTC AACCTCAAGG AGCTGCTGAG ACTGCTTAA GATCTTAA GATCTTAA GATCTTAA GATCTTAA GATCTTAA GATCTTAA
25001	GATCTTAA GATCTTAA GATCTTAA GATCTTAA GATCTTAA GATCTTAA GATCTTAA GATCTTAA GATCTTAA GATCTTAA
25101	AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG
25201	CTCTGCTGAG CTAGGCAACT ACTTGTCTTA CCACTCTTAC ATATGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG
25301	ACCTGCTGAG CTAGGCAACT ACTTGTCTTA CCACTCTTAC ATATGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG
25401	CTCTGCTGAG CTAGGCAACT ACTTGTCTTA CCACTCTTAC ATATGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG
25501	CTCTGCTGAG CTAGGCAACT ACTTGTCTTA CCACTCTTAC ATATGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG
25601	CTCTGCTGAG CTAGGCAACT ACTTGTCTTA CCACTCTTAC ATATGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG AGCTGAGAG

Figure 15P





## pMRKAd5seq MER682

27301	CCGCGCGCGC ACTATCCCGA TCATTTTAT CTATCTTTT AGGTGTTAA GAGCTCGCT GAGCGCTACG ACTGAAATGTT AGTGGGAGAG GCGAGCGAAC
27401	GGAGGCGCGG TGATAGGCTT AGTTAAATTA GGTATTAAT TTCTAGAGCTT CTTGAGCTTC CTGCTGATTC TTACTTAAAC TTCACTGATC GGTCTCTTT
27501	TGCGCCCTGA ACACCTGCTC CACTGTGCTC GGCACATTTT CTCTCTCTT GACTCTGCTG ACTTTTCTTA CTTTGAATTC GGTGAGATTC ATATCTGATC
27601	ACCGGAGCTT TGTGAGACAG GTGACAGCG GGTGTCTAC GAAATCTCG GAAATCTCG GAAATCTCG GAAATCTCG GAAATCTCG GAAATCTCG
27701	CCCGCGCGC GCGCTCGCG TTACCGCGC GCGCTGATC GCGCTGATC GCGCTGATC GCGCTGATC GCGCTGATC GCGCTGATC GCGCTGATC
27801	GGCGCGCGG CCGCGCGCG AGTGGCGCT CCTCTCTGA CCGCGATCG CCGCGATCG CCGCGATCG CCGCGATCG CCGCGATCG CCGCGATCG
27901	CCCTGTGCTC TCACTGTAT TTGCACTGT TTGCACTGT TTGCACTGT TTGCACTGT TTGCACTGT TTGCACTGT TTGCACTGT TTGCACTGT
28001	GGGACACAG AGTCACACTA AGCTTACCA GGAATGAGC CTAAATTAAT TCTAGAAACA CCAATGAGC CCAATGAGC CCAATGAGC CCAATGAGC
28101	GTCTCTATCG CCACTCTCTA AGCTGACCG TTCTGACCG TTCTGACCG TTCTGACCG TTCTGACCG TTCTGACCG TTCTGACCG TTCTGACCG
28201	CGAGGATAGC GGTAGGACAT TTGCGTGCG AGTGGAGCT ACTAGAGAAC CTCTGCGCT GAGAGCTCG AGTGGAGCT ACTAGAGAAC CTCTGCGCT
28301	CAACGCTTTC ACCCGAGCG GATGAGCTT CTACTCAGA CTCTCTCTT CTCTCTCTT CTCTCTCTT CTCTCTCTT CTCTCTCTT CTCTCTCTT
28401	GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT
28501	CCCGGAGCTT ACAGGAGCG CAGCGGCGC AGTGGAGCT AGTGGAGCT AGTGGAGCT AGTGGAGCT AGTGGAGCT AGTGGAGCT AGTGGAGCT
28601	GGCGCGCGG CCGCGCGCG AGTGGAGCT AGTGGAGCT AGTGGAGCT AGTGGAGCT AGTGGAGCT AGTGGAGCT AGTGGAGCT AGTGGAGCT
28701	CAACGCTTTC ACCCGAGCG GATGAGCTT CTACTCAGA CTCTCTCTT CTCTCTCTT CTCTCTCTT CTCTCTCTT CTCTCTCTT CTCTCTCTT
28801	GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT GTGTGAAAT

Figure 15R



## pMRKad5gag MER62

28901 GCGCTACAGC CTGGAAGTCA GGTTCCTTGA ATGTAGCAT CTACTTTTGA CCGAGCACTG TCCGCTGGAT TTTCTCCGT CCAACTACAG CCAACCACTC  
 CCGCATGTGG GAAGTTCAAT CCGTAAAGTGA TACAGTGTGA GACTGAAGCC GGTGCTGTAC AAGGCTCTTA ACAGGTCA GGTGATGC GCTAGGTGAT  
 29001 TACAGAGGAT GACCAACACA ACCAAGCTGG CCGGCTGAC GGTACTTACA TTACACCA ATACACCCCA AGTTCTGCC TTTTCTCAAT ACTGATATA  
 ATTCTCTGA CTGTGTGT TGTGTGGCC GGTGCTGAT GCTTGAATAT ATATATGAT TATGTGGGT TCAGAGCCG TCAAGAGCCG AACACTTAT TCAACCTCAT  
 29101 CTGAGCATG TGTGTGTCT CCAATAGCT CCAATAGCT TATGTGTGA TATGTGTGA TATGTGTGA CATCTCTGC GTAAAGCCCA AACGCTCCCG ACCACCCATC  
 GAACCCGTAC ACCACCAAGA GGTATCCGCA GGTATCCGCA ATACAAATAT ACATATCTA ATACATCTA TATGTGGGT TATGTGGGT TGTGTGTGA TTAATGTGA  
 29201 TATGTGTGA TATGTGTGT ACACCAAGC ATATATCTA TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 ATATATATAT ATATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 29301 CATATATAT CCAATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 29401 GCGCTACAGC CTGGAAGTCA GGTTCCTTGA ATGTAGCAT CTACTTTTGA CCGAGCACTG TCCGCTGGAT TTTCTCCGT CCAACTACAG CCAACCACTC  
 CCGCATGTGG GAAGTTCAAT CCGTAAAGTGA TACAGTGTGA GACTGAAGCC GGTGCTGTAC AAGGCTCTTA ACAGGTCA GGTGATGC GCTAGGTGAT  
 29501 TACAGAGGAT GACCAACACA ACCAAGCTGG CCGGCTGAC GGTACTTACA TTACACCA ATACACCCCA AGTTCTGCC TTTTCTCAAT ACTGATATA  
 ATTCTCTGA CTGTGTGT TGTGTGGCC GGTGCTGAT GCTTGAATAT ATATATGAT TATGTGGGT TCAGAGCCG TCAAGAGCCG AACACTTAT TCAACCTCAT  
 29601 CTGAGCATG TGTGTGTCT CCAATAGCT CCAATAGCT TATGTGTGA TATGTGTGA TATGTGTGA CATCTCTGC GTAAAGCCCA AACGCTCCCG ACCACCCATC  
 GAACCCGTAC ACCACCAAGA GGTATCCGCA GGTATCCGCA ATACAAATAT ACATATCTA ATACATCTA TATGTGGGT TATGTGGGT TGTGTGTGA TTAATGTGA  
 29701 TATGTGTGA TATGTGTGT ACACCAAGC ATATATCTA TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 ATATATATAT ATATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 29801 CATATATAT CCAATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 29901 GCGCTACAGC CTGGAAGTCA GGTTCCTTGA ATGTAGCAT CTACTTTTGA CCGAGCACTG TCCGCTGGAT TTTCTCCGT CCAACTACAG CCAACCACTC  
 CCGCATGTGG GAAGTTCAAT CCGTAAAGTGA TACAGTGTGA GACTGAAGCC GGTGCTGTAC AAGGCTCTTA ACAGGTCA GGTGATGC GCTAGGTGAT  
 30001 TACAGAGGAT GACCAACACA ACCAAGCTGG CCGGCTGAC GGTACTTACA TTACACCA ATACACCCCA AGTTCTGCC TTTTCTCAAT ACTGATATA  
 ATTCTCTGA CTGTGTGT TGTGTGGCC GGTGCTGAT GCTTGAATAT ATATATGAT TATGTGGGT TCAGAGCCG TCAAGAGCCG AACACTTAT TCAACCTCAT  
 30101 CTGAGCATG TGTGTGTCT CCAATAGCT CCAATAGCT TATGTGTGA TATGTGTGA TATGTGTGA CATCTCTGC GTAAAGCCCA AACGCTCCCG ACCACCCATC  
 GAACCCGTAC ACCACCAAGA GGTATCCGCA GGTATCCGCA ATACAAATAT ACATATCTA ATACATCTA TATGTGGGT TATGTGGGT TGTGTGTGA TTAATGTGA  
 30201 TATGTGTGA TATGTGTGT ACACCAAGC ATATATCTA TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 ATATATATAT ATATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 30301 CATATATAT CCAATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT  
 TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT TATATATAT

Figure 155

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30401	AAATTTCTGT CCAATTTATTT CAGCAGCACC TCGTTGCGCTT CCGTCTCACTT CTGCTATTGCG AGCTTCTCCG TCGCTCCAAA CTTCTCTCCAC AATCTAAATG	
30501	TTTAAAGACA GGTCTAAATTA GTGCTGCTGAG AGTAAAGCGA GAAAGCTGGA CAGTAAATAGG TTGAAAGCGG ACCGACGTTT GAAAGAGGTG TTAGATTATAC	
30601	GAATGTCAGT TTCTCTCTGT TCGTCTCCAT CCGTACCCAC TATCTCTCATG TTCTTTTATGA TGAAGCTGCG AATACCGCTCT GAAAGATACCT TCACCCCTCT	
	CTTACACAGTCA AAGGAGGACCA AGGAGAGGTA AGGAGAGGTA CAGTAAATGAG AATACAGTCTT ACTTCTGCGG TTCTGAGCAGA CTTCTATGGA AATGTTGCG A	
	GTATCCATAT GACACGGAATA CCGTCTCTCC ACTCTGCTT TTCTCTTACTG CTCTCTTCTGT ATCTCCCAAT GAGTTCTCAAG AGATCTCCCG TGGGTGACT : TGGGTGACT :	
	CATAGGTATA CTGTGCGCTT GCGCAGGAGG TTGAGACAGGA AAGAGATGAG GAGGGAACA TGGGCTTTA CCGAAGCTTC TCTCAGCGGG ACCCCATGAG	
30701	TCCTTCTGCGG TATCCGAAAC TCTATGTTAC TCTATGCGA TCGTCTGCTT CAAATATGCG AATGATCTT CAGCTGAGCA GCGCGGCAAC CTTTACCTCT	
	AGAAACCGGG ATAGGCTTGG AGATCAATGG AGCTTACCGT ATGAGCGGA GTTTTACCGG TTGCGCGGGA GAGACTGCTT CCGGCGCTTG GAATGAG : GAATGAG :	
30801	AAATGTTAC CACTGTGAG CCACTCTCTA AAATAACCTA GTTAAATATA AATCTGGAAT TATCTGACC CCTCAGATTT ACCTCAGAG CCGTACACTT	
30901	GGCTGCGCGG GCACTCTAA TGTCTGCGGG CACACACTC ACCGTCAAT CACAGCGCC GCTAAACCGG CAGACTGCA AACTTACGAT TGCACCCCA	
	CGACAGCGGG CGTGAGATT ACCAGCGCGG GTTCTGCGGG TTGCTAGCTTA GTCTGCGGG CAGTATGCGA GTCTGAGCT TTGANTGGA ACCTGCGT :	
31001	GGACCGCTCA CAGTGTGGA AGGAAGCTA GCGCTGGA CAGTACCGG CAGTATGCGA GTCTGAGCT TTGANTGGA ACCTGCGT :	
	GTCTGCGGG GTCTAGCTCT TCTTTGAT TCGGCAATG AGGAAGCTA GCGCTGGA CAGTATGCGA GTCTGAGCT TTGANTGGA ACCTGCGT :	
31101	TAACTACTG CACTGTGAG TTGCGCATG TCGGCTTCT CCGTAAATGA GCGCTTAT ACACATGAG GTTCTGATCTT CTTTCTGATG CCGGCAAGTA	
	ATTGATGAG GTGAGCAATG AACCGTAC TGAATTTCT CCGTAAATGA GCGCTTAT ACACATGAG GTTCTGATCTT CTTTCTGATG CCGGCAAGTA	
31201	AGAGAGCTA AACACTTGA CCGTAGCAAC TGTCTGATG GTGCTTAT ATATATCTT CTTGCAACT CTTGCTGAG AAGCTTCTG GAGCTTCTG	
	TCTCTGAT TTGTGAACT GGCATCTTG ACCAGTCTCA CACTGTAAT TATATGAG GAACTTGA TATCTGAG CTTGCTGAG AAGCTTCTG	
31301	CAGGCAATA TGCAGTAA TGTAGCAGGA GTGATCTCT CCGTATCTCT AACTAGAGT TTGCTGAG AACTTCTG TATCTGAG CAGCTTCTA	
	GTCTCTTAT ACTGTAAAT ACATGCTCT CCGTATCTCT AACTAGAGT TTGCTGAG AACTTCTG TATCTGAG CAGCTTCTA	
31401	AACTAAATCT AAGCTAGGA CAGGCGCTC TTTTATATA CTCAGCGCAC AACTTGTATA TTAACTGCA CAGGCGCTT TACTTCTTA CAGCTTCTA	
	TTGATTTAGA TTCTGATCT GTCCCGGGG AATAATTT GAGTCTGCTG TTGATCTAT TCGATCTAT AACTGATCT GTTCTGAG AATGATCTT	
	31501	CAATTCGAAA AAGCTTGAAG TTAACTTAG CACTGCGAG GGTGATGCT TTGAGCTTAC AGCTTCTAC AGCTTCTAC ATTAAAGCA GAGATGCTT
	31601	GTATAGGTTT TTGAGCTCC AATGCGATTC ATTGCGATTC CCGTAACTCA ACTGCGAGT TCGCTATGCG TAAATAGCT CTTCTACCGA ACTTAAAGCA
	31701	TCAGCTAATG CAGCAACAC AATTCGCTC AAACGAAA TTGTGCAATG CTTAGATCTT GATTCAGCA AGCTATGCT TCGTAAAGCA GAAACTGCT
	31801	AGTGGATAC GTGCTTGTG CAGCAACAC AATTCGCTC AAACGAAA TTGTGCAATG CTTAGATCTT GATTCAGCA AGCTATGCT TCGTAAAGCA
	31901	TTAGTTTGA CAGCAACCT GCGTATGAG TCGATTTCT AATATATGAT AAGCTAACT TTGTGCAATG TCGATTTCT TCGTAAAGCA GAAACTGCT
	32001	ATCAGAACT GTGCTTGTG CAGCAACAC AATTCGCTC AAACGAAA TTGTGCAATG CTTAGATCTT GATTCAGCA AGCTATGCT TCGTAAAGCA
	32101	TGCAGAGAAA GATCTTAAAC TCACTTGTG CTTAGCAAAA TGTGCTGCT AATATGATG TCGATTTCT TCGTAAAGCA GAAACTGCT
	32201	ACGCTCTTT CTAGGTTTG AGTGAACCA GAAATGTTTT ACAGCTGAG TTATGAGCG AATGCAAGT CAGCTTCTA CTTAAAGCT TAACTTCTA
	32301	ATATCTGGA CAGTCTGAG TGTCACTCT AATATAGAT TTGCAAAA TGTGCTGCT AATATGATG TCGATTTCT TCGTAAAGCA GAAACTGCT
	32401	TATAGAGCT GTCAAGTTTC AGGATGAGAA TATATCTA AACTGCTTTT ACCTAGCTT TCGATTTCTA TCGATTTCTA TCGATTTCTA
	32501	GAATGAGGA TCTTACTGAA GCGCAAGCTT ATACAAAGC TGTGATTTT AAGCTTCTT TATGCTTCTA TCGATTTCTA TCGATTTCTA
	32601	CTTACCTCT AGATGACTT CCGTCTGGA TATGTTTGG ACACCTAAA TATGATTTG ATAGTCTGAT AGGTTTGA GTGCTTCTT GAGCTTCTT

Figure 15T

pHRIKAD5paq MER602

32101 TACATCTGTC AGTCAGATTT ACTTAACGCG AGCAAAAGCT AAGCTCTGTA CACTAACCTT TACACTAACG GTACACAGCG AMACAGAGCA CACACTCTCA  
ATTGTACACAG TCAGTTCANA TGAATTTTCC TCCTCTTTTA TTCTTACANT GTATATGATA ATGTTATTTG CCATCTCTCT GTCTTACAGT  
32201 AGTCATACCT CTATCTACAT TCTATCTGAC TCTATCTGAT ACAN TACTT TATATGATA TTCTTACANT CTCTTACAGC TTTTCTATAC ATTCTCCANV  
TCAGCTATGA GATACAGTAA ANGTACCTCG ATCAACGCGG TCTCTCTGTA ATTACTTAT ANAGCTCTTA CAGCAATCTG AAAAAATGTA TAACTCTCTT  
32301 AATAAGAAAT CATTCTGTTT ATGTTTCAGC GTCTCTATTT TCAATCTTA GMAATTTTA ATCAATTTT CATTCTAGTG TATATCTCTA CCACCTACATA  
GCTTATCTTA GCAACACAA TCAAAAGTTC CACAAATTA CTTTAAAT CTCTTAAAT TCAATCTTA GTATCTCTAT ATATCTCTCT GTCTCTCTTA  
32401 GCTTATACAG ATCACTCTAC CTTAATCTAA CTTAATCTAA CTTAATCTAA CTTAATCTAA CTTAATCTAA CTTAATCTAA CTTAATCTAA CTTAATCTAA  
GMAATCTCT TAGTCTCTAT GAACTCTCTT GAACTCTCTT GAACTCTCTT GAACTCTCTT GAACTCTCTT GAACTCTCTT GAACTCTCTT GAACTCTCTT  
32501 GCTCTCTCTT AAAAACTCA TATCTCTCTT AACAGCTAA TCTCTCTCTT TATATCTCTT CAGCTCTCTT TCTCTCTCTT TCTCTCTCTT TCTCTCTCTT  
GAACTCTCTT TTTCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
32601 ATAACTCTCT CAGCTCTCTT ACTTATCTCT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
TATCTCTCTT CAGCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT  
32701 AAGCTCTCTT CTAATCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
TCTCTCTCTT GATCTCTCTT CACTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT TATCTCTCTT  
32801 CTTCTCTCTT TACATCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
GATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
32901 TCACTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
AGCTCTCTT TCTCTCTCTT TCTCTCTCTT TCTCTCTCTT TCTCTCTCTT TCTCTCTCTT TCTCTCTCTT TCTCTCTCTT TCTCTCTCTT  
33001 AAGCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
TCTCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
33101 CAGCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
GATCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
33201 AAGCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
TCTCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
33301 CAGCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
GATCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
33401 AAGCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
TCTCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
33501 GAGCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT  
CCTCTCTCTT CAGCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT ATCTCTCTT

Figure 15U

pRRAd5gag MER6B2

33601	CTGAAACAAA ACCAGTGTGG GGGGTGACAA ACAGATCTGG GTCTCTGGTC TTCTGCTTGA GATCCGCTGG TGTAGTAGTT GTATTATATC CACTCTCTTA
33701	GACTTCGTTT TGGTCCAGGC CGGCATCTTT TGTCTATGCG CAGAGTCCAG AGTGGCGATT AGTGGCGATT AGTGGCGATT AGTGGCGATT AGTGGCGATT
33801	AGCATCCAG GGGGCGGCTG GCTTGGGATT CTATTGAAAC TCCCTTATGC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC
33901	TTCTAGGCTC CGCGCGGAC GGAAGGCGAA GATACATTTT AGGATGACG GGAAGGCGAA GGAAGGCGAA GGAAGGCGAA GGAAGGCGAA GGAAGGCGAA
34001	ACCTACACAT TGGTCTGGCG AGTCACACGC GGGAGGAGCG GGAAGGCGAA GGAAGGCGAA GGAAGGCGAA GGAAGGCGAA GGAAGGCGAA GGAAGGCGAA
34101	TGGATGTGTA AGCAAGAGCG TCACTGTGTG CCGTCTCTGC CTTCTCTGAC CTTCTCTGTA CTTCTCTGTA CTTCTCTGTA CTTCTCTGTA CTTCTCTGTA
34201	TTCTCTCTCT TCCGCTCTCC TCCGCTCTCC TCCGCTCTCC TCCGCTCTCC TCCGCTCTCC TCCGCTCTCC TCCGCTCTCC TCCGCTCTCC TCCGCTCTCC
34301	TACTTCTAGA TATTCTACTT GCGCGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG
34401	TCCAAAGGC AAAGCGGCTT CAGGTCCGAG GTGAGGCTAA TGGAGGCTAA TGGAGGCTAA TGGAGGCTAA TGGAGGCTAA TGGAGGCTAA TGGAGGCTAA
34501	AGGTTTCTCG TTTCGCGGGA GTGCGAGGTC ACCTGCATTG ACCTGCATTG ACCTGCATTG ACCTGCATTG ACCTGCATTG ACCTGCATTG ACCTGCATTG
34601	AAATATCTTC ATCTCCGAC CTCTCTATA TATCTCTAAG TATCTCTAAG TATCTCTAAG TATCTCTAAG TATCTCTAAG TATCTCTAAG TATCTCTAAG
34701	TTATTAAAGG TAGAGCGGCG GAAGGCTTAT ATAGAGTTTC ATAGAGTTTC ATAGAGTTTC ATAGAGTTTC ATAGAGTTTC ATAGAGTTTC ATAGAGTTTC
34801	CAGGCTCAGG CAGCGAATCA TGATTCGAAA AATTCAGTTT CTTTCAGTTT CTTTCAGTTT CTTTCAGTTT CTTTCAGTTT CTTTCAGTTT CTTTCAGTTT
34901	GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC
35001	GGTCCCTTGG CAGGCGGAGG TGAACATAAT GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC
35101	CCAGGAGAGC GTCCCGGTCG GCATCTGATTA ACATCTGATG CAGGCTGATG CAGGCTGATG CAGGCTGATG CAGGCTGATG CAGGCTGATG CAGGCTGATG
35201	TATGACAGGC ATACTGCGAG CTATGCTTAC CAGGCTGATG CAGGCTGATG CAGGCTGATG CAGGCTGATG CAGGCTGATG CAGGCTGATG CAGGCTGATG
34401	ATACTGCGAG TATGAGGCTC GATAGGATG GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC GTGCGAGGTC
34501	GGCAAGGCT CGCGGAGGCA AGAGGAGCA TGGTATGAT TGGTATGAT TGGTATGAT TGGTATGAT TGGTATGAT TGGTATGAT TGGTATGAT
34601	CGGTTTCGGA CGCGGTTTTC TCTTCTGCTT TCTTCTGCTT TCTTCTGCTT TCTTCTGCTT TCTTCTGCTT TCTTCTGCTT TCTTCTGCTT TCTTCTGCTT
34701	TCTCAAGCAT GTCTGCGGCT CAGAGCGGCA AAGAGGTTT TGTGTTTAT TGTGTTTAT TGTGTTTAT TGTGTTTAT TGTGTTTAT TGTGTTTAT
34801	AGAGTTTCTTA CAGAGCGGCA AAGAGGTTT TGTGTTTAT TGTGTTTAT TGTGTTTAT TGTGTTTAT TGTGTTTAT TGTGTTTAT TGTGTTTAT
34901	ATAGGAGTAA GAGGAGTAC GAGGAGGCG GGGTACGCTT GGGTACGCTT GGGTACGCTT GGGTACGCTT GGGTACGCTT GGGTACGCTT GGGTACGCTT
35001	TATTCGATTT CTGCTGATG AGACTGAGG AAGAGTACG AAGAGTACG AAGAGTACG AAGAGTACG AAGAGTACG AAGAGTACG AAGAGTACG
35101	AGTATTACAT TCTGAGGAT TGTGAGGAT TGTGAGGAT TGTGAGGAT TGTGAGGAT TGTGAGGAT TGTGAGGAT TGTGAGGAT TGTGAGGAT
35201	AGCAGCATTT ACAGCGGCTA TAGAGGATAT ATGCTGCAAT ATGCTGCAAT ATGCTGCAAT ATGCTGCAAT ATGCTGCAAT ATGCTGCAAT ATGCTGCAAT

Figure 15V

## pMRKad5gug MER682

35301	CATTATTAGA	AACTTACAA	TCCAAACCA	TACAAATAC	TTCCCTTAA	AACTTACCT	ACCCCTCTCC	TTCCCTCTCC	CCGCTCCACG	TTACAAATCT
	GTAAATATCT	TTTGAATGTA	AGGTTTGCT	ATGTTCAAT	AGCTGAT	TTGGATCCG	TTGGCTCCG	AGGCTCTCC	GGCTCTCCG	AGCTTTTGG
35401	CACCCCTCA	TTATCATATT	GGTTCAAT	CANATAGG	TATATTATT	ATATGTTAA	TTAAATAT	GGATCTCCG	GGATCTCCG	GGATCTCCG
	GTGGGGAGT	AATAGTATA	CCGAATTAG	GTATTTATC	ATATATATC	TACTACAA	AACTTTAAG	CTTACAGCT	CTTACAGCT	CTTACAGCT
35501	CCCAATATGA	TTCTTTCTCG	TTCCCTCTCC	ATCCCTATC	CCCTTTCTA	CCCTTTCTA	TTCCCTCTCC	TAGATTTAG	CCCTTTCTA	CCCTTTCTA
	GGTATATAT	AGATAGAGG	AGGCTCTCC	TACCTTAGG	GGCTTTCTA	GGCTTTCTA	AGGCTCTCC	ATCTATCTC	GGTATATAT	GGTATATAT
35601	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
35701	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
35801	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
35901	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36001	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36101	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36201	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36301	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36401	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36501	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36601	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36701	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36801	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
36901	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA
	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA	GGCAGGAAA

Figure 15W

pmrkad5gag MERGB2

37001 CAGACAGGGA TAAATACCGG CCACATACCA GAACTTTTAA AGTCTCTATC ATTCTGAAAC GTTCTTGCGG GCGAAACAC TCAGAGATCT TACTTCTCTT  
GTGTTGCGCT ATTATGCGCG CGGTGTATCT CTTCGAATTT TACAGATGAG TAACTTTTG CAGAGAGCGC CGCTTTTGAG AGTTCTTAGA ATGCKGACAA  
GAGATCCCACT TCGATTGAC CCACTCTGTC ACCCAATCTA TCTTAATAT TCTTAATAT CACTACCTCT TCTGCGTGAG CAAAGACAGC AAGCGCAAT  
CTCTAGGTCA AGCTACATG GTGACCATG TGGTCTGACT AGAATCTCTA GAAATATGAA GTCTCTGCA AGACCCACTC GTTTTGTGCG TTCCCTTTTA  
GCGCGAATAA AGGATATAG GCGGACAGCG AATGCTTGCA TACTATACT GTTCTTTT CAAATATTA GAAACATTTA TCAAGTAT TCTCTCATTA  
CGCGTTTTT TCGCTTATC CGCTCTGTC TTACATAT ATGATATATC GAAATATTA GTTATATGAA CTTCGTAAAT AGTCCGANTA ACAGAGTACT  
GCGGATACAT ATTGATAT ATTATGAAA ATAAACAAAT AGGATTTCTT CTACATTTT CCGCAATGT CCCACCTGAC GTCTAGAGAA CCATTATTA  
CGCTATGTA TAACTTACA TAAATCTTTT TATTGTTTA TCGCCAGCG CGGTATAG CGCTTTTCA CGCTGACTG CAGATTTCTT GGTATATATA

37101

37201

37301

37401 CATGACATTA ACCTATAAA ATAGGCTAT CAGTACGCG TTCTCTCTT AGAATTTGA TCCGAATCT TAAAT (SEQ ID NO: 27)  
GTACTGTAAAT TCGATATTTT TATCCGATA GTGCTCGCG AAGCGAGAG TCTTAACT AGCTTAAGA ATTA (SEQ ID NO: 28)

Band1  
EcdIII

Figure 15X

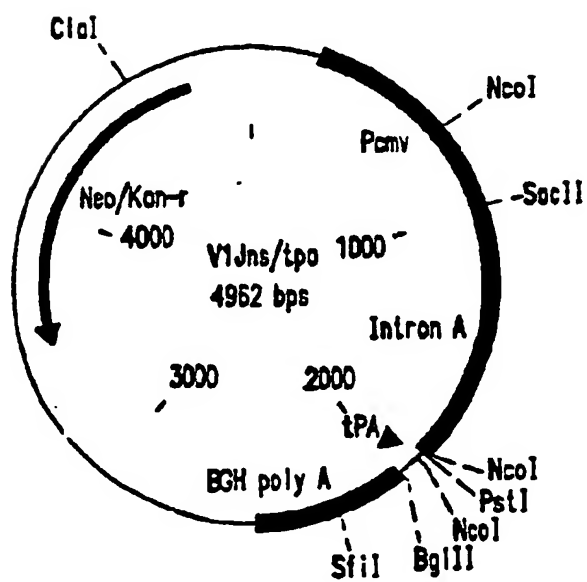
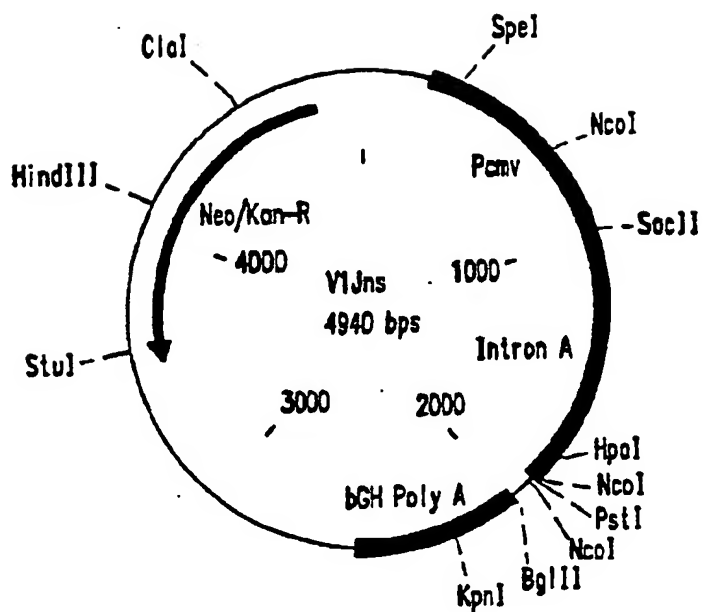


FIGURE 16

AGATCTACCATGGCCCCATCTCCCCATGAGACTGTGCCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGGTGAA  
 Bg/11 MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysVally  
 1 10 20

GCAGTGGCCCCGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA  
 sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL  
 30 40 50

AGATTGGCCCCGAGAACCCTACAACACCCTGTGTTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG  
 ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal  
 60 70

GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCTGGCCTGAAGAA  
 AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLy  
 80 90 100

GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGATGCCTACTTCTGTGCCCCCTGGATGAGGACTTCAGGAAGTACACTG  
 sLysLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA  
 110 120 130

CCTTCACCATCCCTCCATCAACAATGAGACCCCTGGCATCAGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC  
 loPheTrnIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly  
 140 150

TCCCTGCCATCTTCCAGTCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA  
 SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrGl  
 160 170 180

GTACATGGCTGCCCTGTATGTGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACC  
 nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL  
 190 200 210

TGCTGAGGTGGGGCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCTTCTGTGGATGGGCTATGACTGCAC  
 euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis  
 220 230

CCGGACAAGTGGACTGTGCAGCCCATTTGTGCTGCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG  
 ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValGl  
 240 250 260

CAAGCTGAAGTGGGCCTCCAAATCTACCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCC  
 yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaL  
 270 280 290

FIGURE 17A



TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGGAGATCCTGAAGGAGCCTGTGCAT  
E<sup>+</sup>ThrGluValIleProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluProValHis  
300 310

GGGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA  
GlyValTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTy  
320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGCCCCACACCAATGATGTGAAGCAGCTGA  
rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMetArgGlyAlaHisThrAsnAspValLysGlnLeuT  
350 360 370

CTGAGGCTGTGCAGAAGATCACCCTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG  
hrGluAlaValGlnLysIleThrThrGluSerIleValIleTrpGlyLysThrProLysPheLysLeuProIleGlnLys  
380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTGTGAACACCCCCCCCCT  
GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheValAsnThrProProLe  
400 410 420

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCTGTTGGGGCTGAGACCTTCTATCTGGCTGGGGCTGCCAACAGGG  
uValLysLeuTrpTyrGlnLeuGluLysGluProIleValGlyAlaGluThrPheTyrValAlaGlyAlaAlaAsnArgG  
430 440 450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGGAGGCAGGAAGGTGGTGACCCCTGACTGACACCACCAACAG  
luThrLysLeuGlyLysAlaGlyTyrValThrAsnArgGlyArgGlnLysValValThrLeuThrAspThrThrAsnGln  
460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCCTCCAGTATGC  
LysThrAlaLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluValAsnIleValThrAlaSerGlnTyrAl  
480 490 500

CCTGGGCATCATCCAGGCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG  
aLeuGlyIleIleGlnAlaGlnProAspGlnSerGluSerGluLeuValAsnGlnIleIleGluGlnLeuIleLysLysG  
510 520 530

ACAAGGTGTACCTGGCCTGGGTGCCCTGCCACAAGGCCATGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC  
luLysValTyrLeuAlaTrpValProAlaHisLysGlyIleGlyGlyAsnGluGlnValAspLysLeuValSerAlaGly  
540 550

ATCAGGAAGGTGCTGTTCTCGATGGCATTGACAAGCCCCAGGATGAGCATGAGAAGTACCACTCCAACCTGGAGGGCTAT  
IleArgLysValLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMe  
560 570 580

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGGTGGCTAAGGAGATTGTGGCTCCTGTGACAAGTCCAGCTGAAGGGGAGG  
tAlaSerAspPheAsnLeuProProValValAlaLysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluA  
590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCTGGCATCTGGCAGCTGGCCTGCACCCACCTGGAGGGCAAGGTGATCCTGGTG  
IaMetHisGlyGlnValAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysValIleLeuVal  
620 630

GCTGTGCATGTGGCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCTCTGCT  
AlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe  
640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACTGCCAATGGCTCCAACCTCACTGGGGCCACAGTGAGGGCTG  
uLysLeuAlaGlyArgTrpProValLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrValArgAlaA  
670 680 690

CCTGCTGGTGGCTGGCATCAAGCAGGAGTTTGGCATCCCTACAACCCCACTCCAGGGGGTGGTGGCTCCATGAAC  
IaCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyValValAlaSerMetAsn  
700 710

AAGGAGCTGAAGAAGATCATTTGGCAGGTGAGGGACCGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTTCAT  
LysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAlaValPheIle  
720 730 740

CCACAACCTTCAAGAGGAAGGGGGCATCGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC  
eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleG  
750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACCTCAGGGTGTACTACAGGACTCCAGGAACCCCTGTGG  
InThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp  
780 790

AAGGGCCCTGCCAAGCTGCTGTGAAGGGGGAGGGGGCTGTGGTGATCCAGGACAACCTGTGACATCAAGGTGGTGGCCAG  
LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysValValProAr  
800 810 820

GAGGAAGGCCAAGATCATCAGGGACTATGCCAAGCAGATGGCTGGGGATGACTGTGTGGCTCCAGGCAGGATGAGGACT  
gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspx  
830 840 850

AAAGCCCGGCAGATCT (SEQ ID NO: 3)  
Xx BgII (SEQ ID NO: 4)

FIGURE 17C

**FIGURE 18**

WT	- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT	-42
OPT	- ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC M G G K W S K R S V P G W S	-14
WT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT	-84
OPT	- ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC T V R E R M R R A E P A A D	-28
WT	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA	-126
OPT	- AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC R V R R T E P A A V G V G A	-42
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC	-168
OPT	- GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC V S R D L E K H G A I T S S	-56
WT	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA	-210
OPT	- AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC N T A A T N A D C A W L E A	-70
WT	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA	-252
OPT	- CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG Q E D E E V G F P V R P Q V	-84
WT	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC	-294
OPT	- CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC P L R P M T Y K G A V D L S	-98
WT	- CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC	-336
OPT	- CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC H F L K E K G G L E G L I H	-112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC	-378
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC S Q K R Q D I L D L W V Y H	-126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG	-420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC T Q G Y F P D W D N Y T P G	-140

FIGURE 19A

WT	- CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG	-462
OPT	- CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG	
	P G I R F P L T F G W C F K	-154
WT	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA	-504
OPT	- CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG	
	L V P V E P E K V E E A N E	-168
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG	-546
OPT	- GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC	
	G E N N C L L H P M S Q H G	-182
WT	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC	-588
OPT	- ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC	
	I E D P E K E V L E W R F D	-196
WT	- AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG	-630
OPT	- TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC	
	S K L A F H H V A R E L H P	-210
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30)	-651
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9)	
	E Y Y K D C (SEQ ID NO:10)	-216

FIGURE 19B

**FIGURE 20**

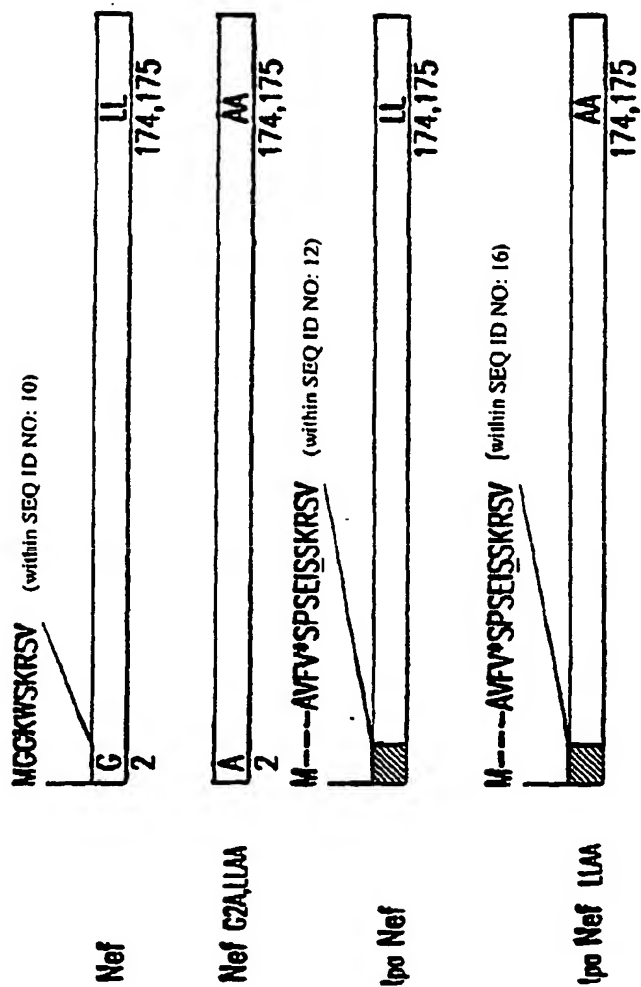


FIGURE 21

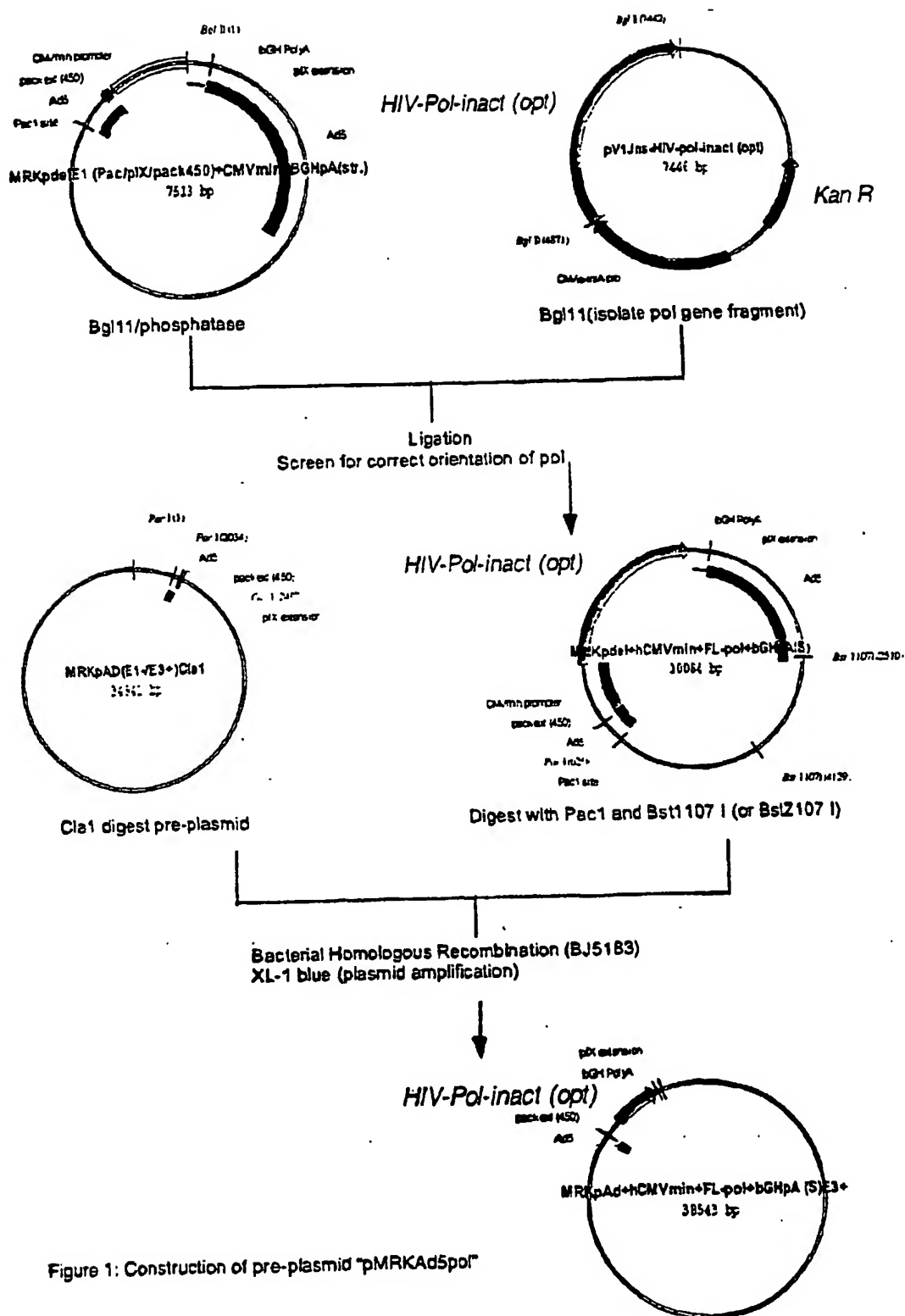


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

FIGURE 22



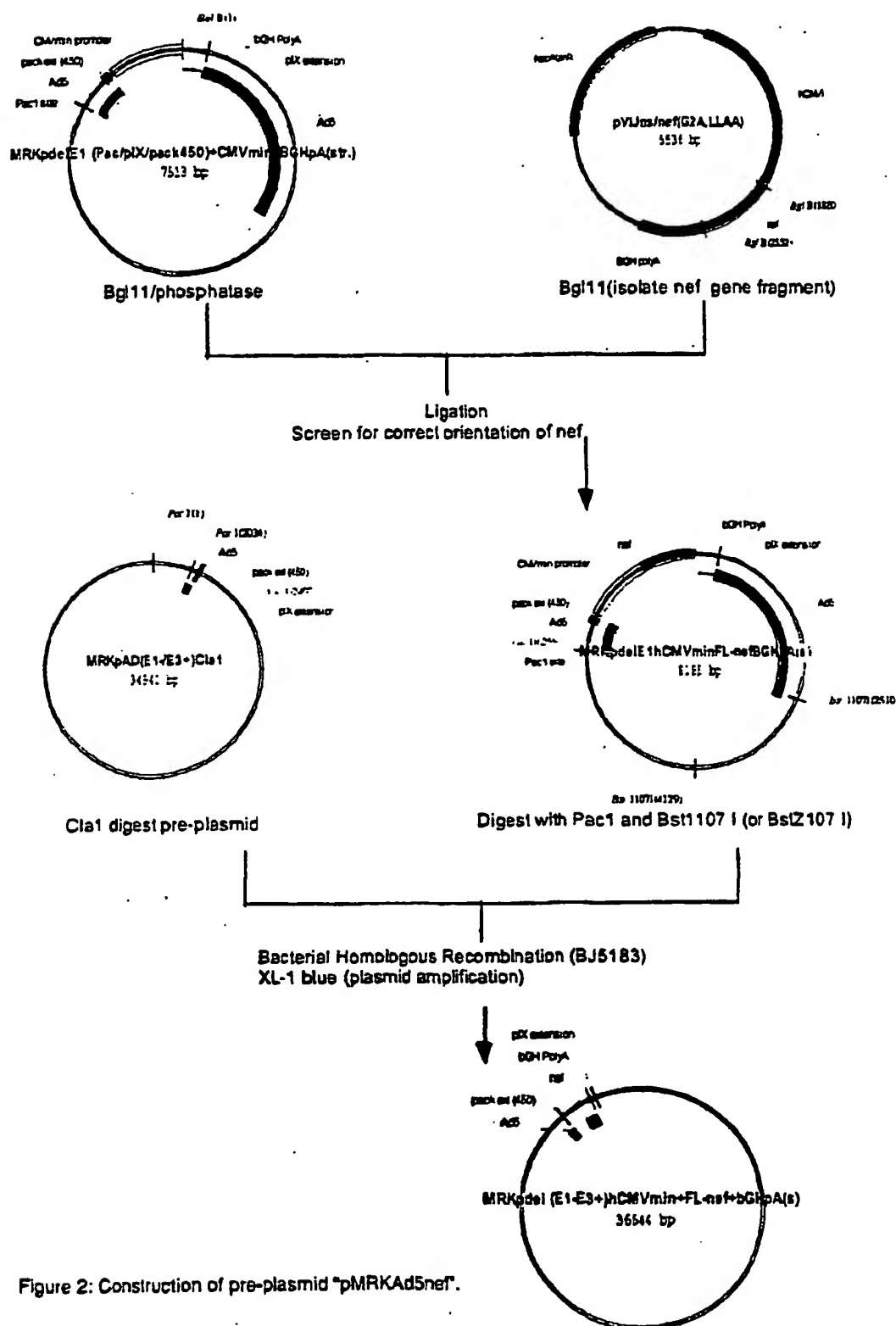
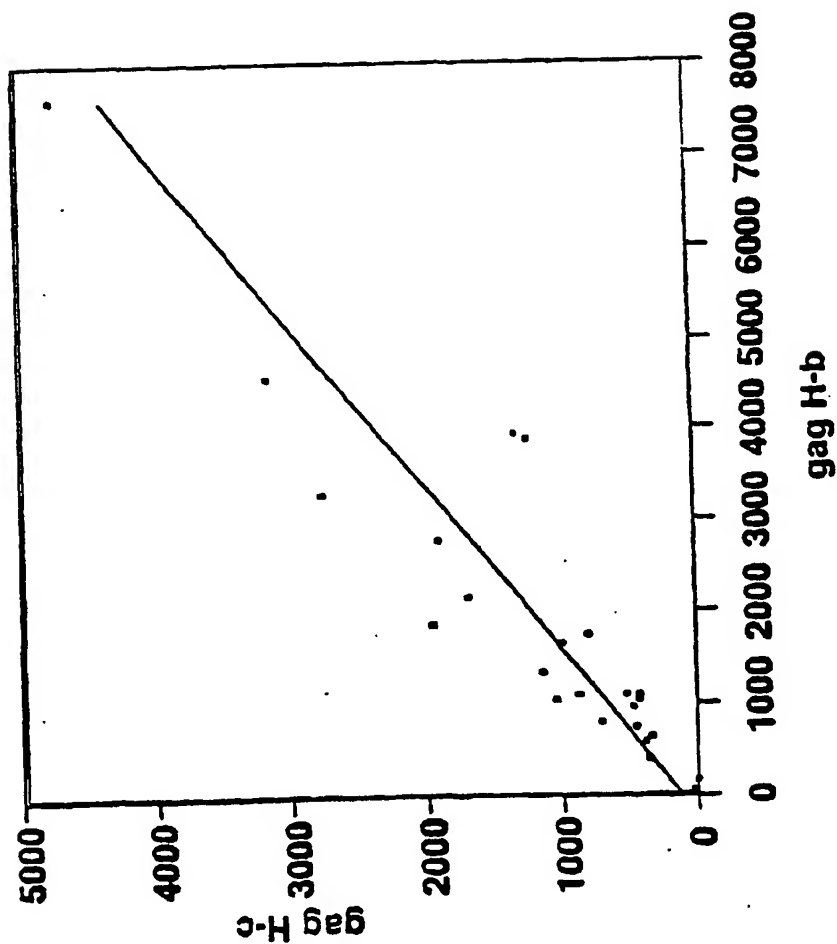


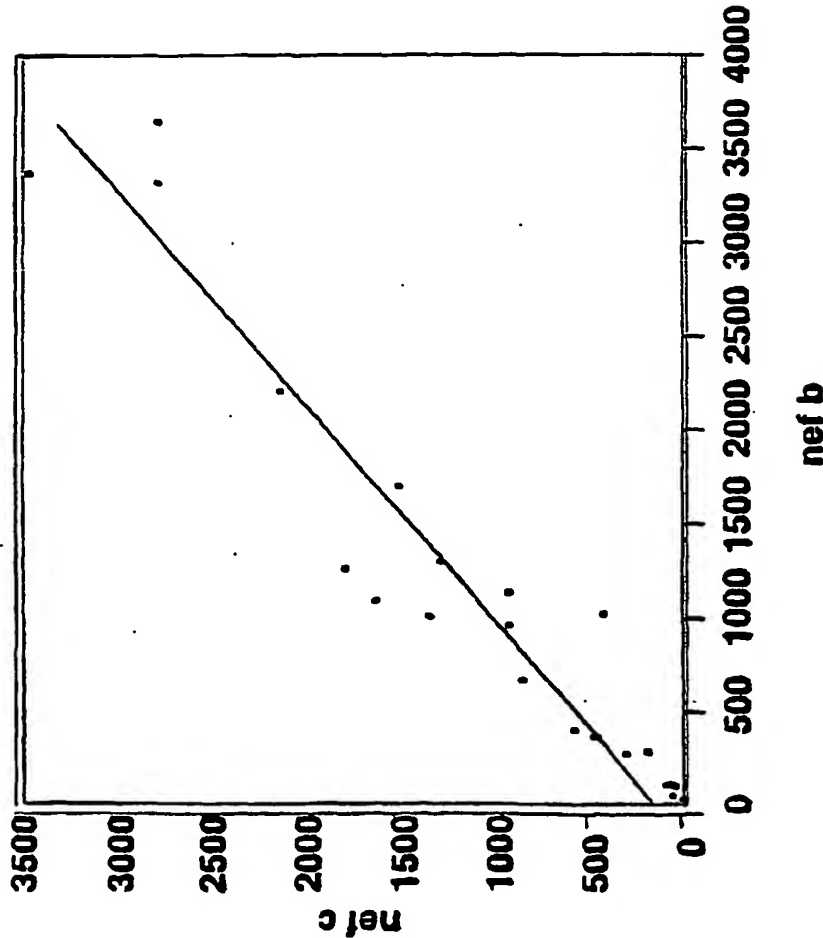
Figure 2: Construction of pre-plasmid "pMRKAd5nef".

FIGURE 23

# Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



**Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects**



$$\text{nef c} = 131.132 + 0.8646 \text{ nef b}$$

Summary of Fit			
RSquare		0.91685	
RSquare Adj		0.91289	
Root Mean Square Error		289.7718	
Mean of Response		1096.435	
Observations (or Sum Wgts)		23	

FIGURE 25

**MRKAd5pol MER1062**  
(MRKAd5 Pre-Adenoviral Vector Containing the LA opt pol Coding Region)

```

1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAACCTAA CTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC GCGCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCTAC ACCGTTTTCA CTGCAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGCGG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAAC TG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACTTTAGA CTTATTAAAA CACATGAGT ATCGCGCATT ATAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGCGCGCC CTGAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCACAAA TAATAATATC

451 GCGGCGCGCA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGCGCCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTACGGT
   TTCCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACGGG CGGACCGTAA TACGGGTCAT

851 CATGACCTTA TGGGACTTTC CTACTGGCA GTACATCTAC GTATTAGTCA
   GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

```

Figure 26A

901 TCGCTATTAC CATGGTGATG CGGTTTGGC AGTACATCAA TGGGCGTGGA  
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA  
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA  
ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG  
TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG  
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTGGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC  
GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT  
AGGCGCCGGC CCTTGCCACG TAACCTTGCG CCTAAGGGG ACGGTTCTCA

1251 GAGATCTACC ATGGCCCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC  
CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG

1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG  
ACTTCGGACC GTACCTACCG GGGTTCCACT TCGTCACCGG GGACTGACTC

1351 GAGAAGATCA AGGCCCTGGT GGAAATCTGC ACTGAGATGG AGAAGGAGGG  
CTCTTCTAGT TCCGGGACEA CCTTTAGACG TGACTCTACC TCTTCCTCCC

1401 CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC CCTGTGTTTG  
GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC

1451 CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG  
GGTAGTTCTT CTTCTGAGG TGGTTCACCT CCTTCGACCA CCTGAAGTCC

1501 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC  
CTCGACTTGT TCTCCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG

1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG  
GGTGGGGCGA CCGGACTTCT TCTTCTCAG AACTGACAC GACCGACACC

1601 GGGATGCCTA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT  
CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTTCATGTGA

1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA  
CGGAAGTGGT AGGGGAGGTA GTTGTTACTC TGGGGACCGT AGTCCATGGT

1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT  
CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACCG TAGAAGGTCA

1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
GGAGGTACTG GTTCTAGGAC CTCGGGAAGT CCTTCGTCTT GGGACTGTAA

1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT  
CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA

Figure 26B

1901 GGGGCCTGAC CACCCCTGAC AAGAAGCACC AGAAGGAGCC CCCCTTCCTG  
CCCCGGACTG GTGGGGACTG TTCTTCGTGG TCTTCCTCGG GGGGAAGGAC

1951 TGGATGGGCT ATGAGCTGCA CCCCACAAAG TGGACTGTGC AGCCCATTTGT  
ACCTACCCGA TACTCGACGT GGGGCTGTTC ACCTGACACG TCGGGTAACA

2001 GCTGCCTGAG AAGGACTCCT GGAAGTGTAA TGACATCCAG AAGCTGGTGG  
CGACGGACTC TTCCTGAGGA CCTGACACTT ACTGTAGGTC TTCGACCACC

2051 GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
CGTTCGACTT GACCCGGAGG GTTTAGATGG GACCGTAGTT CCACTCCGTC

2101 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT  
GACACGTTCG ACGACTCCCC GTGGTTCCGG GACTGACTCC ACTAGGGGGA

2151 GACTGAGGAG GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG  
CTGACTCCTC CGACTCGACC TCGACCGACT CTTGTCCCTC TAGGACTTCC

2201 AGCCTGTGCA TGGGGTGTAC TATGACCCCT CCAAGGACCT GATTGCTGAG  
TCGGACACGT ACCCCACATG ATACTGGGGA GGTTCCTGGA CTAACGACTC

2251 ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC  
TAGGTCTTCG TCCCGGTCCC GGTACCTGG ATGGTTTGA TGGTCTCCGG

2301 CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCACA  
GAAGTTCTTG GACTTCTGAC CGTTCATACG GTCCTACTCC CCCC GGTTGT

2351 CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
GGTTACTACA CTTGTCGAC TGACTCCGAC ACGTCTTCTA GTGGTGACTC

2401 TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA  
AGGTAACACT AGACCCCGTT CTGGGGGTTT AAGTTCGACG GGTAGGTCTT

2451 GGAGACCTGG GAGACCTGGT GGAAGTGAAG CTGGCAGGCC ACCTGGATCC  
CCTCTGGACC CTCTGGACCA CCTGACTCAT GACCGTCCGG TGGACCTAGG

2501 CTGAGTGGGA GTTTGTGAAC ACCCCCCCCC TGGTGAAGCT GTGGTACCAG  
GACTCACCTT CAAACACTTG TGGGGGGGGG ACCACTTCGA CACCATGGTC

2551 CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG ACCTTCTATG TGGCTGGGGC  
GACCTCTTCC TCGGGTAACA CCCCCGACTC TGGAAGATAC ACCGACCCCG

2601 TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG  
ACGGTTGTCC CTCTGGTTCC ACCCGTTCCG ACCGATACAC TGGTTGTCCC

2651 GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
CGTCCGTCTT CCACCACTGG GACTGACTGT GGTGGTTGGT CTTCTGACGG

2701 CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT  
GAGGTCCGGT AGATGGACCG GGAGGTCTTG AGACCGGACC TCCACTTGTG

2751 TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC  
ACACTGACGG AGGGTCATAC GGGACCCGTA GTAGGTCCGG GTCGGACTAG

Figure 26 C

2851 GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA  
CTCTTCCACA TGGACCGGAC CCACGGACGG GTGTTCCCGT AACCCCCGTT

2901 TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC  
ACTCGTCCAC CTGTTGACAC ACAGACGACC GTAGTCCTTC CACGACAAGG

2951 TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
ACCTACCGTA ACTGTTCCGG GTCCTACTCG TACTCTTCAT GGTGAGGTTG

3001 TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA  
ACCTCCCGAT ACCGGAGACT GAAGTTGGAC GGGGGACACC ACCGATTCTT

3051 GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG  
CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC

3101 GGCAGGTGGA CTGCTCCCCCT GGCATCTGGC AGCTGGCCTG CACCCACCTG  
CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC

3151 GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA  
CTCCCCGTTC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAAC

3201 GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC  
CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG

3251 TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG

3301 TCCAATTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT  
AGGTTGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA

3351 CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG  
GTTCTGCTTC AAACCGTAGG GGATGTTGGG GGTCAGGGTC CCCCACCACC

3401 CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG  
GGAGGTACTT GTTCTCGAC TTCTTCTAGT AACCCTGCCA CTCCCTGGTC

3451 GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACCT  
CGACTCGTGG ACTTCTGTG ACACGTCTAC CGACACAAGT AGGTGTTGAA

3501 CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG  
GTTCTCCTTC CCCCCGTAGC CCCCAGATGAG GCGACCCCTC TCCTAACACC

3551 ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
TGTAAGTAACG GTGTCTGTAG GTCTGGTTC TCGAGGTCTT CGTCTAGTGG

3601 AAGATCCAGA ACTTCAGGGT GACTACAGG GACTCCAGGA ACCCCCTGTG  
TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCCT TGGGGGACAC

3651 GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC  
CTTCCCGGGA CGGTTGACG ACACCTTCCC CCTCCCCCGA CACCACTAGG

3701 AGGACAATC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC  
TCCTGTTGAG ACTGTAGTTC CACCACGGGT CCTCCTTCCG GTTCTAGTAG

2 june 26 D

3801 GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAGC  
CCTACTCCTG ATTTTCGGGCC CGTCTAGACG ACACGGAAGA TCAACGGTGC

3851 CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC  
GTAGACAACA AACGGGGAGG GGGCACGGAA GGAAC TGGGA CCTTCCACGG

3901 ACTCCCACTG TCCTTTCTTA ATAAAATGAG GAAATTGCAT CGCATTGTCT  
TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA

3951 GAGTAGGTGT CATCTATTTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG  
CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTCC

4001 GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT  
CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA

4051 ATGGCCGATC GGC CGCCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG  
TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTCCCAC

4101 GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTTGC  
CCTTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG

4151 AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTTGATGGA AGCATTGTGA  
TCGTCGGCGG CGGCGGTACT CGTGGTTGAG CAAACTACCT TCCTAACACT

4201 GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAAT  
CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA

4251 GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCCCG CAAACTCTAC  
CACTACCCGA GGTCGTAACT ACCAGCGGGG CAGGACGGGC GTTTGAGATG

4301 TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGCCT  
ATGGAAGTGG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCGGA

4351 CCGCCGCCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC  
GGCGGCGGCG AAGTCGGCGA CGTCGGTGGC GGGCGCCCTA ACACTGACTG

4401 TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCCGC  
AAACGAAAGG ACTCGGGCGA ACGTTTGTCA CGTCGAAGGG CAAGTAGGCG

4451 CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC  
GGCGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTGGG

4501 GGGAACTTAA TGTGTTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGTT  
CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTGCTCCAA

4551 TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAATAA  
AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTATT

4601 AAAACCAGAC TCTGTTTGGG TTTGGATCAA GCAAGTGTCT TGCTGTCTTT  
TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA

4651 ATTTAGGGGT TTTGCGCGCG CGGTAGGCCG GGGACCAGCG GTCTCGGTGC  
TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTCGC CAGAGCCAGC

Figure 26E



4751 GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCCT  
 CAAGTCTATG TACCCGTATT CGGGCAGAGA CCCCACCTCC ATCGTGGTGA  
 4801 GCAGAGCTTC ATGCTGCGGG GTGGTGTGT AGATGATCCA GTCGTAGCAG  
 CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC  
 4851 GAGCGCTGGG CGTGGTGCCT AAAAATGTCT TTCAGTAGCA AGCTGATTGC  
 CTCGCGACCC GCACCACGGA TTTTACAGA AAGTCATCGT TCGACTAACG  
 4901 CAGGGGCAGG CCCTTGGTGT AAGTGTTTAC AAAGCGGTTA AGCTGGGATG  
 GTCCCCGTCC GGAACCCACA TTCACAAATG TTTCGCCAAT TCGACCCTAC  
 4951 GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTTAGGTTG  
 CCACGTATGC ACCCTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC  
 5001 GCTATGTTCC CAGCCATATC CCTCCGGGGA TTCATGTTGT GCAGAACCAC  
 CGATACAAGG GTCGGTATAG GGAGGCCCCCT AAGTACAACA CGTCTTGGTG  
 5051 CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTCATGT AGCTTAGAAG  
 GTCGTGTCAC ATAGGCCACG TGAACCCCTT AAACAGTACA TCGAATCTTC  
 5101 GAAATGCGTG GAAGAACTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC  
 CTTTACGCAC CTTCTTGAAC CTCTCGGGGA AACTGGAGG TTCTAAAGG  
 5151 ATGCATTCTG CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC  
 TACGTAAGCA GGTATTACTA CCGTTACCCG GTGCCCCGCC GCCGGACCCG  
 5201 GAAGATATTT CTGGGATCAC TAACGTCATA GTTGTTGTTCC AGGATGAGAT  
 CTTCTATAAA GACCCTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA  
 5251 CGTCATAGGC CATTTTACAA AAGCGCGGGC GGAGGGTGCC AGACTGCGGT  
 GCAGTATCCG GTAAAAATGT TTCGCGCCCG CCTCCCACGG TCTGACGCCA  
 5301 ATAATGGTTC CATCCGGCCC AGGGGCGTAG TTACCCCTCAC AGATTTGCAT  
 TATTACCAAG GTAGGCCGGG TCCCCGCATC AATGGGAGTG TCTAAACGTA  
 5351 TTCCCACGCT TTGAGTTCAG ATGGGGGGAT CATGTCTACC TCGGGGGCGA  
 AAGGGTGCGA AACTCAAGTC TACCCCCCTA GTACAGATGG ACGCCCCGCT  
 5401 TGAAGAAAAC GGTTCCTGGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG  
 ACTTCTTTTG CCAAAGGCCC CATCCCCTCT AGTCGACCCT TCTTTCGTCC  
 5451 TTCCTGAGCA GCTGCGACTT ACCGCAGCCG GTGGGCCCCG AAATCACACC  
 AAGGACTCGT CGACGCTGAA TGGCGTCGGC CACCCGGGCA TTTAGTGTGG  
 5501 TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCAGCTG CCGTCATCCC  
 ATAATGGCCG ACGTTGACCA TCAATTCTCT CGACGTCGAC GGCAGTAGGG  
 5551 TGAGCAGGGG GGCCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTC  
 ACTCGTCCCC CCGGTGAAGC AATTCTGTACA GGGACTGAGC GTACAAAAGG  
 5601 CTGACCAAAT CCGCCAGAAG GCGCTCGCCG CCCAGCGATA GCAGTTCTTG  
 GACTGGTTTA GGCGGTCTTC CGCGAGCGGC GGGTCGCTAT CGTCAAGAAC

Figure 26 F

5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCACACAG CTCGGTCACC  
AAAACTCGCA AACTGGTTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG

5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG  
ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC

5801 CGGCTTTTCG TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT  
GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA

5851 CATGTCTTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTCACGG  
GTACAGAAAG GTGCCCGCGT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC

5901 TGAAGGGGTG CGCTCCGGGC TGC CGCTGG CCAGGGTGCG CTTGAGGCTG  
ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCCACGC GAACTCCGAC

5951 GTCCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCCTGCG CGTCGGCCAG  
CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGGTC

6001 GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGCCCTT  
CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACC GGGA

6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCAGA  
ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT

6101 CTTTGTAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA  
GAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGGCCCTCAT

6151 GGCATCCGCG CCGCAGGCCC CGCAGACGGT CTCGCATTCC ACGAGCCAGG  
CCGTAGGCGC GCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC

6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTCCTCCC ATGCTTTTTG  
ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAAC

6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GCTCGGTGAC  
TACGCAAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG

6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCTCGA  
CTTTTCCGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT

6351 GCGGTGTTCC GCGGTCTTCC TCGTATAGAA ACTCGGACCA CTCTGAGACA  
CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT

6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG  
TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTACCC TCCCCATCGC

6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTGA AGACACATGT  
CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACT TCTGTGTACA

6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG  
CGGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGGTGC

6551 TGACCGGGTG TTCCTGAAGG GGGGCTATAA AAGGGGTGG GGGCGCGTTC  
ACTGGCCAC AAGGACTTCC CCCCAGATATT TTCCCCACC CCCGCGCAAG

Figure 266

6651 AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCAGTT  
TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATTC TAACAGTCAA

6701 TCCAAAACG AGGAGGATTT GATATTCACC TGGCCCGCGG TGATGCCTTT  
AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGAAA

6751 GAGGGTGGCC GCATCCATCT GGTCAGAAAA GACAATCTTT TTGTTGTCAA  
CTCCCACCGG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACAAACAGTT

6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTTGGCGATG  
CGAACCACCG TTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC

6851 GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCGCGAT  
CTCGCGTCCC AAACCAAAAA CAGCGCTAGC GCGCGAGGA ACCGGCGCTA

6901 GTTTAGCTGC ACGTATTCGC GCGCAACGCA CCGCCATTCT GGAAAGACGG  
CAAATCGACG TGCATAAGCG CGCGTTGCGT GCGGTAAGC CCTTCTGCCC

6951 TGGTGCGCTC GTCGGGCACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG  
ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTTGGCGC CAACACGTCC

7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT  
CACTGTTCCA GTTGCACCA CCGATGGAGA GCGCATCCG CGAGCAACCA

7051 CCAGCAGAGG CGGCCGCCCT TGC GCGAGCA GAATGGCGGT AGGGGTCTA  
GGTCGTCTCC GCCGGCGGGA ACGCGCTCGT CTTACCGCCA TCCCCAGAT

7101 GCTGCGTCTC GTCCGGGGGG TCTGCGTCCA CGGTAAAGAC CCCGGGCAGC  
CGACGCAGAG CAGGCCCCCC AGACGCAGGT GCCATTCTG GGGCCCGTCG

7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCAT CTTGCAAGT CTAGCGCCTG  
TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC

7201 CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC  
GACGTTACGC GCCCGCCGTT CGCGCGCGAG CATACCCAAC TCACCCCTG

7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTGG  
GGGTACCGTA CCCACCCAC TCGCGCCTCC GCATGTACGG CGTTTACAGC

7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT  
ATTTGCATCT CCCCAGAGAGA CTCATAAGGT TCTATACATC CCATCGTAGA

7351 TCCACGCGG ATGCTGGCGC GCACGTAATC GTATAGTTCTG TCGAGGGAG  
AGGTGGCGCC TACGACCGCG CGTGCATTAG CATATCAAGC ACGCTCCCTC

7401 CGAGGAGGTC GGGACCGAGG TTGCTACGGG CGGGCTGCTC TGCTCGGAAG  
GCTCCTCCAG CCTGGCTCC AACGATGCCC GCCCGACGAG ACGAGCCTTC

7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG  
TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC

7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCCTCA CGCACGAAGG  
CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC

Figure 26 H

7601 TCTAGGGCGC AGTAGTCCAG GGTTCCTTG ATGATGTCAT ACTTATCCTG  
 AGATCCCGCG TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC  
 7651 TCCCTTTTTT TTCCACAGCT CGCGGTTGAG GACAACTCT TCGCGGTCTT  
 AGGGAAGAAA AAGGTGTCGA GCGCCAACTC CTGTTTGAGA AGCGCCAGAA  
 7701 TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT  
 AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCTCGGA  
 7751 AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC  
 TCGTACATCT TGACCAACTG CCGGACCATC CGCGTCGTAG GGAAAAGATG  
 7801 GGGTAGCGCG TATGCCTGCG CGGCCTTCCG GAGCGAGGTG TGGGTGAGCG  
 CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC  
 7851 CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG  
 GTTCCACAG GGACTGGTAC TGAAACTCCA TGACCATAAA CTTCACTCAC  
 7901 TCGTCGCATC CGCCCTGCTC CCAGAGCAA AAGTCCGTGC GCTTTTTGGA  
 AGCAGCGTAG GCGGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAAACCT  
 7951 ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTTCCCG  
 TGCGCCATAA CCGTCCCGCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC  
 8001 CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGGTCCCGG CACCTCGGAA  
 GCGCTCCGTA TTTCAACGCA CACTACGCCT TCCCAGGGCC GTGGAGCCTT  
 8051 CGGTTGTTAA TTACCTGGGC GCGGAGCAG ATCTCGTCAA AGCCGTTGAT  
 GCCAACAAAT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA  
 8101 GTTGTGGCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG  
 CAACACCGGG TGTTACATTT CAAGGTTCTT CGCGCCCTAC GGGAACTACC  
 8151 AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC  
 TTCCGTTAAA AAATCAAGG AGCATCCACT CGAGAAGTCC CCTCGACTCG  
 8201 CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA  
 GGCACGAGAC TTTCCCGGGT CAGACGTTCT ACTCCCAACC TTCGCTGCTT  
 8251 TGAGCTCCAC AGGTACCGGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG  
 ACTCGAGGTG TCCAGTGCCC GGTAAATCGTA AACGTCCACC AGCGCTTTCC  
 8301 TCCTAAACTG GCGACCTATG GCCATTTTTT CTGGGGTGAT GCAGTAGAAG  
 AGGATTTGAC CGCTGGATAC CGGTAAAAAA GACCCCACTA CGTCATCTTC  
 8351 GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTTCG CGGCTAGGTC  
 CATTGCCCCA GAACAAGGGT CGCCAGGGTA GGTTCCAAGC GCCGATCCAG  
 8401 TCGCGCGGCA GTCAGTAGAG GCTCATCTCC GCCGAACTTC ATGACCAGCA  
 AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CGGCTTGAAG TACTGGTCTG  
 8451 TGAAGGGCAC GAGCTGCTTC CCAAAGGCCC CCATCCAAGT ATAGGTCTCT  
 ACTTCCCGTG CTCGACGAAG GGTTCGCGG GGTAGGTTCA TATCCAGAGA

Figure 26I

8551 GAAGAACTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT  
CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCGAT AACTACACCA

8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTAA  
CTTTCATCTT CAGGGACGCT GCCCGGCTTG TGAGCACGAC CGAAAACATT

8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG  
TTTGACACGG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC

8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCCCT  
CAACTGGACT GCTGGCGCGT GTTCCTTCGT CTCACCCTTA AACTCGGGGA

8751 CGCCTGGCGG GTTTGGCTGG TGGTCTTCTA CTTGCGCTGC TTGTCCTTGA  
GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAAC

8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG  
GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGCGC

8851 CGAGCCCAAA GTCCAGATGT CCGCGCGCGG CGGTCGGAGC TTGATGACAA  
GCTCGGGTTT CAGGTCTACA GGCGCGCGCC GCCAGCCTCG AACTACTGTT

8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGGCGTCAGG  
GTAGCGCGTC TACCCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC

8951 TCAGGCGGGA GCTCCTGCAG GTTTACCTCG CATAGACGGG TCAGGGCGCG  
AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCCGCGC

9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT  
CCGATCTAGG TCCACTATGG ATTAAAGGTC CCCGACCAAC CACCGCCGCA

9051 CGATGGCTTG CAAGAGGCCG CATCCCCGCG GCGCGACTAC GGTACCGCGC  
GCTACCGAAC GTTCTCCGGC GTAGGGGCGC CGCGCTGATG CCATGGCGCG

9101 GGCGGGCGGT GGGCCGCGGG GGTGTCCTTG GATGATGCAT CTAAAAGCGG  
CCGCCCCGCA CCCGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCC

9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG  
ACTGCGCCCG CTCGGGGGCC TCCATCCCCC CCGAGGCCTG GGCGGCCCTC

9201 AGGGGGCAGG GGCACGTCGG CGCCGCGCGC GGGCAGGAGC TGGTGCTGCG  
TCCCCCGTCC CCGTGCAGCC GCGGCGCGCG CCCGTCTCG ACCACGACGC

9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GGCGGTTGAT CTCCTGAATC  
GCGCATCCAA CGACCGCTTG CGCTGCTGCG CCGCCAATA GAGGACTTAG

9301 TGGCGCCTCT GCGTGAAGAC GACGGGCCCC GTGAGCTTGA ACCTGAAAGA  
ACCGCGGAGA CGCACTTCTG CTGCCCCGGC CACTCGAACT TGGACTTTCT

9351 GAGTTCGACA GAATCAATTT CGGTGTCGTT GACGGCGGCC TGGCGCAAAA  
CTCAAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCGCCGG ACCGCGTTTT

9401 TCTCCTGCAC GTCTCCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC  
AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J

9501 GCGGCGGAGG TCGTTGGAAA TCGGGGCCAT GAGCTGCGAG AAGGCGTTGA  
CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT

9551 GGCCTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG  
CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGG AAGCCGTAGC

9601 CGGGCGCGCA TGACCACCTG CGCGAGATTG AGCTCCACGT GCCGGGCGAA  
GCCCCGCGCT ACTGGTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCCGTT

9651 GACGGCGTAG TTTCGCAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG  
CTGCCGCATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC

9701 TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGGATTCTG  
ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC

9751 TTGATATCCC CCAAGGCCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC  
AACTATAGGG GGTTCGCGAG TTCCGCGAGG TACCGGAGCA TCTTCAGGTG

9801 GGCGAAGTTG AAAAAGTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT  
CCGCTTCAAC TTTTGTACCC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA

9851 CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG  
GGTCTTCTGC CTACTCGAGC CGCTGTCACA GCGCGTGGAG CGCGAGTTTC

9901 GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC  
CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGGAG

9951 CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC  
GGGAAGAAGA AGAAGACCGC CGCCACCCCC TCCCCCTGT GCCGCGCTG

10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG  
CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC

10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG  
GCTGCCGCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCCGCGTC

10101 TTGGAAGACG CCGCCCGTCA TGTCCCGGTT ATGGGTTGGC GGGGGGCTGC  
AACCTTCTGC GGCGGGCAGT ACAGGGCCAA TACCCAACCG CCCCCGACG

10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA  
GTACGCCGTC CCTATGCCGC GATTGCTACG TAGAGTTGTT AACAACACAT

10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA  
CCATGAGGCG GCGGCTCCCT GGAATCGCTC AGGCGTAGCT GGCCTAGCCT

10251 AAACCTCTCG AGAAAGGCGT CTAACCAGTC ACAGTCGCAA GGTAGGCTGA  
TTTGAGAGC TCTTCCGCA GATTGGTCAG TGTCAGCGTT CCATCCGACT

10301 GCACCGTGGC GGGCGGCAGC GGGCGGCGGT CGGGGTTGTT TCTGGCGGAG  
CGTGGCACCG CCCGCCGTCG CCCGCCGCA GCCCAACAA AGACCGCCTC

10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GGCGGATGGT  
CACGACGACT ACTACATTAA TTTCATCCGC CAGAACTCTG CCGCCTACCA

Figure 26 K

10451 CGGCCATGCC CCAGGCTTCG TTTTGACATC GGCGCAGGTC TTTGTAGTAG  
GCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AACATCATC

10501 TCTTGCAATGA GCCTTTCTAC CGGCACCTCT TCTTCTCCTT CCTCTTGTCC  
AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG

10551 TGCATCTCTT GCATCTATCG CTGCGGCGGC GGCGGAGTTT GGCCGTAGGT  
ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCCTCAAA CCGGCATCCA

10601 GGCGCCCTCT TCCTCCCATG CGTGTGACCC CGAAGCCCTT CATCGGCTGA  
CCGCGGGAGA AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT

10651 AGCAGGGCTA GGTGCGGCAC AACCGGCTCG GCTAATATGG CCTGCTGCAC  
TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG

10701 CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CCGTGGTATG  
GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATAC

10751 CGCCCGTGTT GATGGTGTA GTGCAGTTGG CCATAACGGA CCAGTTAACG  
GCGGGCACAA CTACCACATT CACGTCAACC GGTATTGCCT GGTCAATTGC

10801 GTCTGGTGAC CCGGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC  
CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG

10851 CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC  
GGAGCTCAGT TTATGCATCA GCAACGTTCA GGCGTGGTCC ATGACCATAG

10901 CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG  
GGTGGTTTTT CACGCCGCCG CCGACCGCCA TCTCCCCGGT CGCATCCCAC

10951 GCCGGGGCTC CGGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA  
CGGCCCGGAG GCGGCCGCTC TAGAAGGTTG TATTCCGCTA CTATAGGCAT

11001 GATGTACCTG GACATCCAGG TGATGCCGGC GGCGGTGGTG GAGGCGCGCG  
CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCCGCGCGC

11051 GAAAGTCGCG GACGCGGTTC CAGATGTTGC GCAGCGGCAA AAAGTGCTCC  
CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTCACGAGG

11101 ATGGTCGGGA CGCTCTGGCC GGTCAGGCGC GCGCAATCGT TGACGCTCTA  
TACCAGCCCT GCGAGACCGG CCAGTCCGCG CCGGTTAGCA ACTGCGAGAT

11151 GACCGTGCAA AAGGAGAGCC TGTAAGCGGG CACTCTTCCG TGGTCTGGTG  
CTGGCACGTT TTCTCTCGG ACATTGCCCC GTGAGAAGGC ACCAGACCAC

11201 GATAAATTCG CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA  
CTATTTAAGC GTTCCCATAG TACCGCCTGC TGGCCCCAAG CTCGGGGCAT

11251 TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGCGT GTCGAACCCA  
AGGCCGGCAG GCGGCACTAG GTACGCCAAT GGCGGGCGCA CAGCTTGGGT

11301 GGTGTGCGAC GTCAGACAAC GGGGGAGTGC TCCTTTTGGC TTCCTTCCAG  
CCACACGCTG CAGTCTGTTG CCCCCACAG AGGAAAACCG AAGGAAGGTC

Figure 26L

11401 AAGCGGTTAG GCTGGAAAGC GAAAGCATT AAGTGGCTCGC TCCCTGTAGC  
TTCGCCAATC CGACCTTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG

11451 CGGAGGGTTA TTTTCCAAGG GTTGAGTCGC GGGACCCCCG GTTCGAGTCT  
GCCTCCCAAT AAAAGGTTCC CAACTCAGCG CCCTGGGGGC CAAGCTCAGA

11501 CGGACCGGCC GGACTGCGGC GAACGGGGGT TTGCCTCCCC GTCATGCAAG  
GCCTGGCCGG CCTGACGCCG CTTGCCCCCA AACGGAGGGG CAGTACGTTT

11551 ACCCCGCTTG CAAATTCCTC CGGAAACAGG GACGAGCCCC TTTTGTGCTT  
TGGGGCGAAC GTTAAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAAACGAA

11601 TTCCAGATG CATCCGGTGC TCGGGCAGAT GCGCCCCCTT CCTCAGCAGC  
AAGGTCTAC GTAGGCCACG ACGCCGTCTA CGCGGGGGGA GGAGTCGTCT

11651 GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCCTC CCCTCCTCCT  
CCGTCTCTCGT TCTCGTCGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA

11701 ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA  
TGGCGCAGTC CTCCCCGCTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT

11751 TTACGAACCC CCGCGGCGCC GGGCCCGGCA CTACCTGGAC TTGGAGGAGG  
AATGCTTGGG GCGCGCCGCG CCCGGGCGGT GATGGACCTG AACCTCCTCC

11801 GCGAGGGCCT GCGCGCGCTA GGAGCGCCCT CTCCTGAGCG GCACCCAAGG  
CGCTCCCGGA CCGCGCCGAT CCTCGCGGGA GAGGACTCGC CGTGGGTTCC

11851 GTGCAGCTGA AGCGTGATAC GCGTGAGGCG TACGTGCCGC GGCAGAACCT  
CACGTCGACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGGA

11901 GTTTCGCGAC CGCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT  
CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA

11951 TCCACGCAGG GCGCGAGCTG CGGCATGGCC TGAATCGCGA GCGGTTGCTG  
AGGTGCGTCC CCGGCTCGAC GCCGTACCGG ACTTAGCGCT CGCCAACGAC

12001 CGCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG  
GCGCTCCTCC TGAAACTCGG GCTGCGCGCT TGGCCCTAAT CAGGGCGCGC

12051 CGCACACGTG GCGGCCGCGG ACCTGGTAAC CGCATAACGAG CAGACGGTGA  
GCGTGTGCAC CGCCGGCGGC TGGACCATTG GCGTATGCTC GTCTGCCACT

12101 ACCAGGAGAT TAACCTTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT  
TGGTCTCTTA ATTGAAAGTT TTTTCGAAAT TGTTGGTGCA CGCATGCGAA

12151 GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT  
CACCGCGCGC TCCTCCACCG ATATCCTGAC TACGTAGACA CCCTGAAACA

12201 AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCCGCTCATG GCGCAGCTGT  
TTCGCGCGAC CTCGTTTGG GTTTATCGTT CGGCGAGTAC CCGTCTGACA

12251 TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTAG GGATGCGCTG  
AGGAATATCA CGTCGTGTCT TCCCTGTTGC TCCGTAAGTC CCTACGCGAC

Figure 26 M



12351 CCTGCAGAGC ATAGTGGTGC AGGAGCGCAG CTTGAGCCTG GCTGACAAGG  
GGACGTCTCG TATCACCACG TCCTCGCGTC GAACTCGGAC CGACTGTTCC

12401 TGGCCGCCAT CAACTATTCC ATGCTTAGCC TGGGCAAGTT TTACGCCCCG  
ACCGCGGGTA GTTGATAAGG TACGAATCGG ACCCGTTCAA AATGCGGGCG

12451 AAGATATACC ATACCCCTTA CGTTCCCAT A GACAAGGAGG TAAAGATCGA  
TTCTATATGG TATGGGGAAT GCAAGGGTAT CTGTTCTCTCC ATTTCTAGCT

12501 GGGGTTCTAC ATGCGCATGG CGCTGAAGGT GCTTACCTTG AGCGACGACC  
CCCCAAGATG TACGCGTACC GCGACTTCCA CGAATGGAAC TCGTGCTGG

12551 TGGGCGTTTA TCGCAACGAG CGCATCCACA AGGCCGTGAG CGTGAGCCGG  
ACCCGCAAAT AGCGTTGCTC GCGTAGGTGT TCCGGCACTC GCACTCGGCC

12601 CGGCGCGAGC TCAGCGACCG CGAGCTGATG CACAGCCTGC AAAGGGCCCT  
GCCGCGCTCG AGTCGCTGGC GCTCGACTAC GTGTCGGACG TTTCCCGGGA

12651 GGCTGGCAGC GGCAGCGGCG ATAGAGAGGC CGAGTCCTAC TTTGACGCGG  
CCGACCGTGC CCGTCGCCGC TATCTCTCCG GCTCAGGATG AAAGTGCGCC

12701 GCGCTGACCT GCGCTGGGCC CCAAGCCGAC GCGCCCTGGA GGCAGCTGGG  
CGCGACTGGA CGCGACCCGG GGTTCGGCTG CGCGGGACCT CCGTCGACCC

12751 GCCGGACCTG GGCTGGCGGT GGCACCCGCG CGCGCTGGCA ACGTCGGCGG  
CGGCCTGGAC CCGACCGCCA CCGTGGGCGC GCGCGACCGT TGCAGCCGCC

12801 CGTGGAGGAA TATGACGAGG ACGATGAGTA CGAGCCAGAG GACGGCGAGT  
GCACCTCCTT ATACTGCTCC TGCTACTCAT GCTCGGTCTC CTGCCGCTCA

12851 ACTAAGCGGT GATGTTTCTG ATCAGATGAT GCAAGACGCA ACGGACCCGG  
TGATTGCGCA CTACAAAGAC TAGTCTACTA CGTTCTGCGT TGCCTGGGCC

12901 CGGTGCGGGC GGCCTGTCAG AGCCAGCCGT CCGGCCTTAA CTCCACGGAC  
GCCACGCCCCG CCGCGACGTC TCGGTGCGCA GGCCGGAATT GAGGTGCTTG

12951 GACTGGCGCC AGGTCATGGA CCGCATCATG TCGCTGACTG CGCGCAATCC  
CTGACCGCGG TCCAGTACCT GCGGTAGTAC AGCGACTGAC GCGCGTTAGG

13001 TGACCGGTTT CCGCAGCAGC CGCAGGCCAA CCGGCTCTCC GCAATTCTGG  
ACTGCGCAAG GCCGTCGTCG GCGTCCGGTT GGCCGAGAGG CGTTAAGACC

13051 AAGCGGTGGT CCCGGCGCGC GCAAACCCCA CGCACGAGAA GGTGCTGGCG  
TTCGCCACCA GGGCCGCGCG CGTTTGGGGT GCGTGCTCTT CCACGACCGC

13101 ATCGTAAACG CGCTGGCCGA AAACAGGGCC ATCCGGCCCG ACGAGGCCGG  
TAGCATTTGC GCGACCGGCT TTTGTCCCGG TAGGCCGGGC TGCTCCGGCC

13151 CCTGGTCTAC GACGCGCTGC TTCAGCGCGT GGCTCGTTAC AACAGCGGCA  
GGACCAGATG CTGCGCGACG AAGTCGCGCA CCGAGCAATG TTGTCGCCGT

13201 ACGTGCAGAC CAACCTGGAC CGGCTGGTGG GGGATGTGCG CGAGGCCGTG  
TGCACGTCTG GTTGGACCTG GCCGACCACC CCCTACACGC GCTCCGGCAC

Figure 26 N

13301 ACTAAACGCC TTCCTGAGTA CACAGCCCGC CAACGTGCCG CGGGGACAGG  
TGATTTGCGG AAGGACTCAT GTGTCGGGCG GTTGACGCGC GCCCCTGTC

13351 AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA  
TCCTGATGTG GTTGAAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT

13401 CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTTT TCCAGACCAG  
GGCGTTTCAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTCTGGTC

13451 TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAAAGTTCG  
ATCTGTTCCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTTGAACG

13501 AGGGGCTGTG GGGGGTGCGG GCTCCACAG GCGACCGCGC GACCGTGCTT  
TCCCCGACAC CCCCCACGCC CGAGGGTGTC CGCTGGCGCG CTGGCACAGA

13551 AGCTTGCTGA CGCCCAACTC GCGCCTGTTG CTGCTGCTAA TAGCGCCCTT  
TCGAACGACT GCGGGTTGAG CGCGGACAAC GACGACGATT ATCGCGGGAA

13601 CACGGACAGT GGCAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA  
GTGCCTGTCA CCGTCGCACA GGGCCCTGTG TATGGATCCA GTGAACGACT

13651 CACTGTACCG CGAGGCCATA GGTCAGGCGC ATGTGGACGA GCATACTTTC  
GTGACATGGC GCTCCGGTAT CCAGTCCGCG TACACCTGCT CGTATGAAAG

13701 CAGGAGATTA CAAGTGTCAG CCGCGCGCTG GGGCAGGAGG ACACGGGCAG  
GTCTCTAAT GTTCACAGTC GGCAGCGGAC CCCGTCTTCC TGTGCCCGTC

13751 CCTGGAGGCA ACCCTAAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC  
GGACCTCCGT TGGGATTTGA TGGACGACTG GTTGGCCGCC GTCTTCTAGG

13801 CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG  
GGAGCAACGT GTCAAATTG TCGTCTCTCC TCGCGTAAAA CGCGATGCAC

13851 CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGGTAA CGCCCAGCGT  
GTCGTCTCGC ACTCGGAATT GGAATACGCG CTGCCCCATT GCGGGTCGCA

13901 GCGCGTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCTCAA  
CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCCGTAC ATACGGAGTT

13951 ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTGATCGC CGCGCCGCC  
TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG

14001 GTGAACCCCG AGTATTTTAC CAATGCCATC TTGAACCCGC ACTGGCTACC  
CACTTGGGGC TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG

14051 GCCCCCTGGT TTCTACACCG GGGGATTCGA GGTGCCCGAG GGTAACGATG  
CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCATTGCTAC

14101 GATTCTCTG GGACGACATA GACGACAGCG TGTTTTCCCC GCAACCGCAG  
CTAAGGAGAC CCTGCTGTAT CTGCTGTCGC ACAAAGGGG CGTTGGCGTC

14151 ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTGCGAAA  
TGGGACGATC TCAACGTTGT CCGCTCGTC CGTCTCCGCC GCGACGCTTT

Figure 260

14251 CGCGGTCAGA TGCTAGTAGC CCATTTCCAA GCTTGATAGG GTCTCTTACC  
GCGCCAGTCT ACGATCATCG GGTAAAGGTT CGAACTATCC CAGAGAATGG

14301 AGCACTCGCA CCACCCGCCC GCGCCTGCTG GGCGAGGAGG AGTACCTAAA  
TCGTGAGCGT GGTGGGCGGG CGCGGACGAC CCGCTCCTCC TCATGGATTT

14351 CAACTCGCTG CTGCAGCCGC AGCGCGAAAA AAACCTGCCT CCGGCATTTT  
GTTGAGCGAC GACGTCGGCG TCGCGCTTTT TTTGGACGGA GGCCGTAAAG

14401 CCAACAACGG GATAGAGAGC CTAGTGGACA AGATGAGTAG ATGGAAGACG  
GGTTGTTGCC CTATCTCTCG GATCACCTGT TCTACTCATC TACCTTCTGC

14451 TACGCGCAGG AGCACAGGGA CGTGCCAGGC CCGCGCCCGC CCACCCGTCG  
ATGCGCGTCC TCGTGTCCTT GCACGGTCCG GGCGCGGGCG GGTGGGCAGC

14501 TCAAAGGCAC GACCGTCAGC GGGGTCTGGT GTGGGAGGAC GATGACTCGG  
AGTTTCCGTG CTGGCAGTCG CCCCAGACCA CACCCTCCTG CTA CTGAGCC

14551 CAGACGACAG CAGCGTCCTG GATTTGGGAG GGAGTGGCAA CCCGTTTGCG  
GTCTGCTGTC GTCGCAGGAC CTAAACCCTC CCTCACCGTT GGGCAAACGC

14601 CACCTTCGCC CCAGGCTGGG GAGAATGTTT TAAAAAATAA AAAAGCATGA  
GTGGAAGCGG GGTCCGACCC CTCTTACAAA ATTTTTTTTT TTTTCGTACT

14651 TGCAAAATAA AAAACTCACC AAGGCCATGG CACCGAGCGT TGTTTCTT  
ACGTTTTATT TTTTGAGTGG TTCCGGTACC GTGGCTCGCA ACCAAAAGAA

14701 GTATTCCTCT TAGTATGCGG CGCGCGGCGA TGTATGAGGA AGGTCTCTCT  
CATAAGGGGA ATCATACGCC GCGCGCCGCT ACATACTCCT TCCAGGAGGA

14751 CCCTCCTACG AGAGTGTGGT GAGCGCGGCG CCAGTGGCGG CGGCGCTGGG  
GGGAGGATGC TCTCACACCA CTCGCGCCGC GGTCACCGCC GCCGCGACCC

14801 TTCTCCCTTC GATGCTCCCC TGGACCCGCC GTTTGTGCCT CCGCGGTACC  
AAGAGGGAAG CTACGAGGGG ACCTGGGCGG CAAACACGGA GGCGCCATGG

14851 TGCGGCCTAC CGGGGGGAGA AACAGCATCC GTTACTCTGA GTTGGCACCC  
ACGCCGGATG GCCCCCTCT TTGTCGTAGG CAATGAGACT CAACCGTGGG

14901 CTATTCGACA CCACCCGTGT GTACCTGGTG GACAACAAGT CAACGGATGT  
GATAAGCTGT GGTGGGCACA CATGGACCAC CTGTTGTTCA GTTGCTTACA

14951 GGCATCCCTG AACTACCAGA ACGACCACAG CAACTTTCTG ACCACGGTCA  
CCGTAGGGAC TTGATGGTCT TGCTGGTGTG GTTGAAAGAC TGGTGCCAGT

15001 TTCAAAACAA TGACTACAGC CCGGGGGAGG CAAGCACACA GACCATCAAT  
AAGTTTTGTT ACTGATGTCT GGCCCCCTCC GTTCGTGTGT CTGGTAGTTA

15051 CTTGACGACC GGTGCACTG GGGCGGCGAC CTGAAAACCA TCCTGCATAC  
GAACTGCTGG CCAGCGTGAC CCCGCCGCTG GACTTTTGGT AGGACGTATG

15101 CAACATGCCA AATGTGAACG AGTTCATGTT TACCAATAAG TTTAAGGCGC  
GTTGTACGGT TTACACTTGC TCAAGTACAA ATGGTTATTC AAATTCGCGC

Figure 26 P

15151 GGGTGATGGT GTCGCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTGAAA  
CCCCTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT

15201 TACGAGTGGG TGGAGTTCAC GCTGCCCCGAG GGCAACTACT CCGAGACCAT  
ATGCTCAGCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA

15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG  
CTGGTATCTG GAATACTTGT TGCCTAGCA CCTCGTGATG AACTTTCACC

15301 GCAGACAGAA CGGGGTCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC  
CGTCTGTCTT GCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG

15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG  
GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC

15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT  
CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAC GACGGTCCTA

15451 GCGGGGTGGA CTTACCCAC AGCCGCTGA GCAACTTGTT GGGCATCCGC  
CGCCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG

15501 AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA  
TTCGCCGTTG GGAAGGTCCT CCCGAAATCC TAGTGGATGC TACTAGACCT

15551 GGGTGGTAAAC ATTCCCGCAC TGTGATGT GGACGCTAC CAGGCGAGCT  
CCCACCATG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA

15601 TGAAAGATGA CACCGAACAG GCGGGGGTG GCGCAGGCGG CAGCAACAGC  
ACTTTCTACT GTGGCTTGTC CCGCCCCAC CGCGTCCGCC GTCGTTGTGC

15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA  
TCACCGTCGC CGCGCCTTCT CTTGAGGTTG CGCCGTCGGC GCCGTTACGT

15701 GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA  
CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT

15751 CACGGGCTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC  
GTGCCGACT CCTCTTCGCG CGACTCCGGC TTCGTCGCCG GCTTCGACGG

15801 GCCCCCGCTG CGCAACCCGA GGTGAGAAG CCTCAGAAGA AACCGGTGAT  
CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA

15851 CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA  
GTTTGGGGAC TGTCTCCTGT CGTTCTTTGC GTCAATGTTG GATTATTCGT

15901 ATGACAGCAC CTTACCCAG TACCGCAGCT GGTACCTTGC ATACAACTAC  
TACTGTCGTG GAAGTGGGTC ATGGCGTCGA CCATGGAACG TATGTTGATG

15951 GCGACCCCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA  
CCGCTGGGAG TCTGGCCTTA GCGAGTACC TGGGACGAAA CGTGAGGACT

16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC  
GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG

16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG  
TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

Figure 26 Q

16151 GGCCGTCTAC TCCCAACTCA TCCGCCAGTT TACCTCTCTG ACCCAGCTGT  
CCGGCAGATG AGGGTTGAGT AGGCGGTCAA ATGGAGAGAC TGGGTGCACA

16201 TCAATCGCTT TCCCGAGAAC CAGATTTTGG CGCGCCCGCC AGCCCCCACC  
AGTTAGCGAA AGGGCTCTTG GTCTAAAACC GCGCGGGCGG TCGGGGGTGG

16251 ATCACCACCG TCAGTGAAAA CGTTCTCTGCT CTCACAGATC ACGGGACGCT  
TAGTGGTGGC AGTCACTTTT GCAAGGACGA GAGTGTCTAG TGCCCTGCGA

16301 ACCGCTGCGC AACAGCATCG GAGGAGTCCA GCGAGTGACC ATTACTGACG  
TGGCGACGCG TTGTCGTAGC CTCCTCAGGT CGCTCACTGG TAATGACTGC

16351 CCAGACGCCG CACCTGCCCC TACGTTTACA AGGCCCTGGG CATAGTCTCG  
GGTCTGCGGC GTGGACGGGG ATGCAAATGT TCCGGGACCC GTATCAGAGC

16401 CCGCGCGTCC TATCGAGCCG CACTTTTGA GCAAGCATGT CCATCCTTAT  
GGCGCGCAGG ATAGCTCGGC GTGAAAACT CGTTCTGTACA GGTAGGAATA

16451 ATCGCCACGC AATAACACAG GCTGGGGCCT GCGCTTCCCA AGCAAGATGT  
TAGCGGGTCG TTATTGTGTC CGACCCCGGA CGCGAAGGGT TCGTTCTACA

16501 TTGGCGGGGC CAAGAAGCGC TCCGACCAAC ACCCAGTGCG CGTGCGCGGG  
AACCGCCCCG GTTCTTCGCG AGGCTGGTTG TGGGTCACGC GCACGCGCCC

16551 CACTACCGCG CGCCCTGGGG CGCGCACAAA CGCGGCCGCA CTGGGCGCAC  
GTGATGGCGC GCGGGACCCC GCGCGTGTTC GCGCCGGCGT GACCCGCGTG

16601 CACCGTCGAT GACGCCATCG ACGCGGTGGT GGAGGAGGCG CGCAACTACA  
GTGGCAGCTA CTGCGGTAGC TGCGCCACCA CCTCCTCCGC GCGTTGATGT

16651 CGCCACGCC GCCACCACTG TCCACAGTGG ACGCGGCCAT TCAGACCGTG  
GCGGGTGCGG CGGTGGTCAC AGGTGTCACC TGCGCCGGTA AGTCTGGCAC

16701 GTGCGCGGAG CCCGGCGCTA TGCTAAAATG AAGAGACGGC GGAGGCGCGT  
CACGCGCCTC GGGCCGCGAT ACGATTTTAC TTCTCTGCCG CCTCCGCGCA

16751 AGCACGTCGC CACCGCCGCC GACCCGGCAC TGCCGCCCAA CGCGCGGCGG  
TCGTGCAGCG GTGGCGGCGG CTGGGCGGTG ACGGCGGGTT GCGCGCCGCC

16801 CGGCCCTGCT TAACCGCGCA CGTCGCACCG GCCGACGGC GGCCATGCGG  
GCCGGGACGA ATTGGCGCGT GCAGCGTGGC CGGCTGCCCG CCGGTACGCC

16851 GCGCTCGAA GGCTGGCCGC GGGTATTGTC ACTGTGCCCC CCAGGTCCAG  
CGGCGAGCTT CCGACCGGCG CCCATAACAG TGACACGGGG GGTCCAGGTC

16901 GCGACGAGCG GCCCGCGCAG CAGCCGCGGC CATTAGTGCT ATGACTCAGG  
CGCTGCTCGC CGGCGGCGTC GTCGGCGCCG GTAATCACGA TACTGAGTCC

16951 GTCGCAGGGG CAACGTGTAT TGGGTGCGCG ACTCGGTTAG CGGCCTGCGC  
CAGCGTCCCC GTTGACACATA ACCCAGCGCG TGAGCCAATC GCCGGACGCG

17001 GTGCCCCTGC GCACCCGCC CCCGCGCAAC TAGATTGCAA GAAAAAATA  
CACGGGCACG CGTGGGCGGG GGGCGCGTTG ATCTAACGTT CTTTTTTGAT

Figure 26 R

17101 CTATGTCCAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG  
 GATACAGGTT CCGGTTTTAG TTTCTTCTCT ACGAGGTCCA GTAGCGCGCG

17151 GAGATCTATG GCGCCCCGAA GAAGGAAGAG CAGGATTACA AGCCCCGAAA  
 CTCTAGATAC CGGGGGGCTT CTTCTTCTC GTCTAATGT TCGGGGCTTT

17201 GCTAAAGCGG GTCAAAAAGA AAAAGAAAGA TGATGATGAT GAACTTGACG  
 CGATTTTCGCC CAGTTTTTCT TTTCTTTCT ACTACTACTA CTGAACTGC

17251 ACGAGGTGGA ACTGCTGCAC GCTACCGCGC CCAGGCGACG GGTACAGTGG  
 TGCTCCACCT TGACGACGTG CGATGGCGCG GGTCCGCTGC CCATGTCACC

17301 AAAGGTGCGAC GCGTAAACG TGTTTTGCGA CCCGGCACCA CCGTAGTCTT  
 TTTCCAGCTG CGCATTTTGC ACAAACGCT GGGCCGTGGT GGCATCAGAA

17351 TACGCCCCGT GAGCGCTCCA CCCGCACCTA CAAGCGCGTG TATGATGAGG  
 ATGCGGGCCA CTCGCGAGGT GGGCGTGGAT GTTCGCGCAC ATACTACTCC

17401 TGTACGGCGA CGAGGACCTG CTTGAGCAGG CCAACGAGCG CCTCGGGGAG  
 ACATGCCGCT GTCCTGGAC GAACTCGTCC GGTGCTCGC GGAGCCCCTC

17451 TTTGCCTACG GAAAGCGGCA TAAGGACATG CTGGCGTTGC CGCTGGACGA  
 AAACGGATGC CTTTCGCCGT ATTCCTGTAC GACCGCAACG GCGACCTGCT

17501 GGGCAACCCA ACACCTAGCC TAAAGCCCGT AACACTGCAG CAGGTGCTGC  
 CCCGTTGGGT TGTGGATCGG ATTTCCGGCA TTGTGACGTC GTCCACGACG

17551 CCGCGCTTGC ACCGTCCGAA GAAAAGCGCG GCCTAAAGCG CGAGTCTGGT  
 GGGCGGAACG TGGCAGGCTT CTTTTCGCGC CGGATTTGCG GCTCAGACCA

17601 GACTTGGCAC CCACCGTGCA GCTGATGGTA CCCAAGCGCC AGCGACTGGA  
 CTGAACCGTG GGTGGCACGT CGACTACCAT GGGTTCGCGG TCGCTGACCT

17651 AGATGTCTTG GAAAAATGA CCGTGGAACC TGGGCTGGAG CCCGAGGTCC  
 TCTACAGAAC CTTTTTTACT GGCACCTTGG ACCCGACCTC GGGTCCAGG

17701 GCGTGCGGCC AATCAAGCAG GTGGCGCCGG GACTGGGCGT GCAGACCGTG  
 CGCACGCCGG TTAGTTCGTC CACCGCGGCC CTGACCCGCA CGTCTGGCAC

17751 GACGTTTCTG TACCCACTAC CAGTAGCACC AGTATTGCCA CCGCCACAGA  
 CTGCAAGTCT ATGGGTGATG GTCATCGTGG TCATAACGGT GGGGTTGTCT

17801 GGGCATGGAG ACACAAACGT CCCCAGTTGC CTCAGCGGTG GCGGATGCCG  
 CCCGTACCTC TGTGTTTGCA GGGGCCAACG GAGTCGCCAC CGCTACGGC

17851 CCGTGCAGGC GGTGCTGCG GCCGCGTCCA AGACCTCTAC GGAGGTGCAA  
 GCCACGTCCG CCAGCGACGC CGGCGCAGGT TCTGGAGATG CCTCCACGTT

17901 ACGGACCCGT GGATGTTTCG CGTTTCAGCC CCCCAGCGCC CGCGCCGTTT  
 TGCCTGGGCA CCTACAAAGC GCAAAGTCGG GGGGCCGCGG GCGCGCAAG

17951 GAGGAAGTAC GCGCCGCCA GCGCGCTACT GCCGAATAT GCCCTACATC  
 CTCCTTCATG CCGCGGCGGT CGCGCGATGA CGGGCTTATA CGGGATGTAG

Figure 26S

18051 AGACGAGCAA CTACCCGACG CCGAACCACC ACTGGAACCC GCCGCCGCCG  
TCTGCTCGTT GATGGGCTGC GGCTTGGTGG TGACCTTGGG CGGCGGCGGC

18101 TCGCCGTCGC CAGCCCGTGC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC  
AGCGGCAGCG GTCGGGCACG ACCGGGGCTA AAGGCACGCG TCCCACCGAG

18151 GCGAAGGAGG CAGGACCCTG GTGCTGCCAA CAGCGCGCTA CCACCCCAGC  
CGCTTCCTCC GTCCTGGGAC CACGACGGTT GTCGCGCGAT GGTGGGGTCC

18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCCTCACCTG  
TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAC

18251 CCGCCTCCGT TTCCCGGTGC CGGGATTCCG AGGAAGAATG CACCGTAGGA  
GGCGGAGGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT

18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TCGCACCAC  
CCCCGTACCG GCCGGTGCCG GACTGCCCCG CGTACGCAGC ACGCGTGGTG

18351 CGGCGGCGGC GCGCGTCGCA CCGTCGCATG CGCGGCGGTA TCCTGCCCCCT  
GCCGCCGCCG CGCGCAGCGT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA

18401 CCTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT  
GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA

18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG  
GGCACC GGAA CGTCCGCGTC TCTGTGACTA ATTTTGTTC AACGTACACC

18501 AAAAATCAAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCCTGTAAC  
TTTTTAGTTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG

18551 TATTITGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC  
ATAAACATC TTACCTTCTG TAGTTGAAAC GCAGAGACCG GGGCGCTGTG

18601 GGCTCGCGCC CGTTCATGGG AAACCTGGCA GATATCGGCA CCAGCAATAT  
CCGAGCGCGG GCAAGTACCC TTTGACCGTT CTATAGCCGT GGTCGTTATA

18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAAAAATT  
CTCGCCACCG CGGAAGTCGA CCCCAGCGCA CACCTCGCCG TAATTTTAA

18701 TCGGTTCCAC CGTTAAGAAC TATGGCAGCA AGGCCTGGAA CAGCAGCACA  
AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGGACCTT GTCGTCGTGT

18751 GGCCAGATGC TGAGGGATAA GTTGAAAGAG CAAAATTTC AACAAAAGGT  
CCGGTCTACG ACTCCCTATT CAACTTCTC GTTTTAAAGG TTGTTTTCCA

18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGGTGGAC CTGGCCAACC  
CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTGG

18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCCTCCCCTA  
TCCGTACAGT TTTATTCTAA TTGTCAATCG AACTAGGGGC GGGAGGGCAT

18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GGCCTGGCGA  
CTCCTCGGAG GTGGCCGGCA CCTCTGTAC AGAGGTCTCC CCGCACCGCT

Figure 26T

19001 AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT  
TCGGAGGGAG CATGCTCCTC CGTGATTTCG TTCCGGACGG GTGGTGGGCA

19051 CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CACCCGTAAC  
GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG

19101 GCTGGACCTG CCTCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG  
CGACCTGGAC GGAGGGGGGC GGCTGTGGGT CGTCTTTGGA CACGACGGTC

19151 GCGCGACCGC CGTTGTTGTA ACCCGTCCTA GCGCGCGCTC CCTGCGCCGC  
CGGGCTGGCG GCAACAACAT TGGGCAGGAT CCGCGCGCAG GGACGCGGCG

19201 GCGGCCAGCG GTCCGCGATC GTTGCGGCCC GTAGCCAGTG GCAACTGGCA  
CGGCGGTGCG CAGGCGCTAG CAACGCCGGG CATCGGTCAC CGTTGACCGT

19251 AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC  
TTCGTGTGAC TTGTCTAGC ACCCAGACCC CCACGTTAGG GACTTCGCGC

19301 GACGATGCTT CTGATAGCTA ACGTGTCGTA TGTGTGTCAT GTATGCGTCC  
CTGCTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATACGCAGG

19351 ATGTCGCCGC CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA  
TACAGCGGCG GTCTCCTCGA CGACTCGGCG GCGCGCGGGC GAAAGGTTCT

19401 TGGCTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC  
ACCGATGGGG AAGCTACTAC GGCCTCACCA GAATGTACGT GTAGAGCCCC

19451 CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCAGT TTGCCCGCGC  
GTCTGCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG

19501 CACCGAGACG TACTTCAGCC TGAATAACAA GTTTAGAAAC CCCACGGTGG  
GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC

19551 CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG  
GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAA CTGCGACGCC

19601 TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT  
AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA

19651 CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT  
GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA

19701 TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT  
AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA

19751 GGCCTGCCT ACAACGCCCT GGCTCCCAAG GGTGCCCCAA ATCCTTGCGA  
CCGTGACGGA TGTGCGGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT

19801 ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG  
TACCCTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTTCTCCTGC

19851 ATGACAACGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAAATCAC  
TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTTGTAGTG

Figure 26 u



19951 TCAAATAGGT GTCGAAGGTC AAACACCTAA ATATGCCGAT AAAACATTTC  
AGTTTATCCA CAGCTTCCAG TTTGTGGATT TATACGGCTA TTTTGTAAAG

20001 AACCTGAACC TCAAATAGGA GAATCTCAGT GGTACGAAAC AGAAATTAAT  
TTGGACTTGG AGTTTATCCT CTTAGAGTCA CCATGCTTGT TCTTTAATTA

20051 CATGCAGCTG GGAGAGTCCT AAAAAAGACT ACCCCAATGA AACCATGTTA  
GTACGTCGAC CCTCTCAGGA TTTTCTCTGA TGGGGTACT TTGGTACAAT

20101 CGGTTCATAT GCAAAACCCA CAAATGAAAA TGGAGGGCAA GGCATTCTTG  
GCCAAGTATA CGTTTTGGGT GTTTACTTTT ACCTCCCGTT CCGTAAGAAC

20151 TAAAGCAACA AAATGGAAAG CTAGAAAGTC AAGTGGAAAT GCAATTTTTC  
ATTCGTTGT TTTACCTTTC GATCTTTCAG TTCACCTTTA CGTTAAAAAG

20201 TCAACTACTG AGGCAGCCGC AGGCAATGGT GATAACTTGA CTCCTAAAGT  
AGTTGATGAC TCCGTCGGCG TCCGTTACCA CTATTGAACT GAGGATTTC

20251 GGTATTGTAC AGTGAAGATG TAGATATAGA AACCCAGAC ACTCATATTT  
CCATAACATG TCACTTCTAC ATCTATATCT TTGGGGTCTG TGAGTATAAA

20301 CTTACATGCC CACTATTAAG GAAGGTAAC CACGAGAACT AATGGGCCAA  
GAATGTACGG GTGATAATTC CTTCATTGA GTGCTCTTGA TTACCCGGTT

20351 CAATCTATGC CCAACAGGCC TAATTACATT GCTTTTAGGG ACAATTTTAT  
GTTAGATACG GGTGTCCGG ATTAATGTAA CGAAATCCC TGTTAAAAATA

20401 TGGTCTAATG TATTACAACA GCACGGGTAA TATGGGTGTT CTGGCGGGCC  
ACCAGATTAC ATAATGTTGT CGTGCCCAT ATACCCACAA GACCGCCCGG

20451 AAGCATCGCA GTTGAATGCT GTTGTAGATT TGCAAGACAG AAACACAGAG  
TTCGTAGCGT CAACTTACGA CAACATCTAA ACGTCTGTC TTTGTGTCTC

20501 CTTTCATACC AGCTTTTGCT TGATTCCATT GGTGATAGAA CCAGGTACTT  
GAAAGTATGG TCGAAAACGA ACTAAGGTAA CCACTATCTT GGTCCATGAA

20551 TTCTATGTGG AATCAGGCTG TTGACAGCTA TGATCCAGAT GTTAGAATTA  
AAGATACACC TTAGTCCGAC AACTGTCGAT ACTAGGTCTA CAATCTTAAT

20601 TTGAAATCA TGGAACGTAA GATGAACCTC CAAATTACTG CTTTCCACTG  
AACTTTTAGT ACCTTGACTT CTACTTGAAG GTTTAATGAC GAAAGGTGAC

20651 GGAGGTGTGA TTAATACAGA GACTCTTACC AAGGTAAAAC CTAAAACAGG  
CCTCCACACT AATTATGTCT CTGAGAATGG TTCCATTTTG GATTTTGTCC

20701 TCAGGAAAAT GGATGGGAAA AAGATGCTAC AGAATTTTCA GATAAAAATG  
AGTCCTTTTA CCTACCCTTT TTCTACGATG TCTTAAAAGT CTATTTTTTAC

20751 AAATAAGAGT TGGAAATAAT TTTGCCATGG AAATCAATCT AAATGCCAAC  
TTTATTCTCA ACCTTTATTA AAACGGTACC TTTAGTTAGA TTTACGGTGT

20801 CTGTGGAGAA ATTTCTGTGA CTCCAACATA GCGCTGTATT TGCCCGACAA  
GACACCTCTT TAAAGGACAT GAGGTTGTAT CGCGACATAA ACGGGCTGTT

Figure 26 v

20901 ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGA CTGCTAC  
TGCTGATGTA CTTGTTGCT CACCACCGAG GGCCCGATCA CCTGACGATG

20951 ATTAACCTTG GAGCACGCTG GTCCCTTGAC TATATGGACA ACGTCAACCC  
TAATTGGAAC CTCGTGCGAC CAGGGAAGT ATATACCTGT TGCAGTTGGG

21001 ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG  
TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC

21051 GCAATGGTCTG CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCTTT  
CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA

21101 GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGAA  
CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT

21151 CTTAGGAAG GATGTTAACA TGGTTCTGCA GAGCTCCCTA GGAAATGACC  
GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CTTTACTGG

21201 TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC  
ATTCCCAACT GCCTCGGTG TAATTCAAAC TATCGTAAAC GGAAATGCGG

21251 ACCTTCTTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT  
TGGAAGAAGG GGTACCGGGT GTTGTGGCGG AGGTGCGAAC TCCGGTACGA

21301 TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA  
ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT

21351 ACATGCTCTA CCCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC  
TGTACGAGAT GGGATATGGG CGGTGCGAT GGTGACACGG GTATAGGTAG

21401 CCCTCCCGCA ACTGGGCGGC TTTCCGCGGC TGGGCCTTCA CGCGCCTTAA  
GGGAGGGCGT TGACCCGCCG AAAGGCGCCG ACCCGGAAGT GCGCGGAATT

21451 GACTAAGGAA ACCCCATCAC TGGGCTCGGG CTACGACCCT TATTACACCT  
CTGATTCTT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA

21501 ACTCTGGCTC TATACCCTAC CTAGATGGAA CCTTTTACCT CAACCACACC  
TGAGACCGAG ATATGGGATG GATCTACCTT GGAAATGGA GTTGGTGTGG

21551 TTTAAGAAGG TGGCCATTAC CTTTGA CTCT TCTGTCAGCT GGCCTGGCAA  
AAATTCTTCC ACCGGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT

21601 TGACCGCCTG CTTACCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG  
ACTGGCGGAC GAATGGGGT TGCTCAAAC TTAATTCGCG AGTCAACTGC

21651 GGGAGGGTTA CAACGTTGCC CAGTGTAACA TGACCAAAGA CTGGTTCCTG  
CCCTCCCAAT GTTGCAACGG GTCACATTGT ACTGGTTTCT GACCAAGGAC

21701 GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC  
CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG

21751 AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA  
TCTCTCGATG TTCCTGGCGT ACATGAGGAA GAAATCTTTG AAGGTCGGGT

Figure 26 w

21851 GGCATCCTAC ACCAACACAA CAACTCTGGA TTTGTTGGCT ACCTTGCCCC  
CCGTAGGATG TGGTTGTGTT GTTGAGACCT AAACAACCGA TGGAACGGGG

21901 CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA  
GTGGTACGCG CTTCTGTCC GGATGGGACG ATTGAAGGGG ATAGGCGAAT

21951 TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCTTTGCGAT  
ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTCAA AGAAACGCTA

22001 CGCACCCTTT GCGCATCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC  
GCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG

22051 ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACTCC GCCCACGCGC  
TGAGTGTCTG GACCCGGTTT TGGAAGAGAT GCGGTTGAGG CGGGTGCGCG

22101 TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCAC CCTTCTTTAT  
ATCTGTACTG AAAACTCCAC CTAGGGTACC TGCTCGGGTG GGAAGAAATA

22151 GTTTTGTTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACC GCGG  
CAAAACAAAC TTCAGAACT GCACCAGGCA CACGTGGTCG GCGTGGCGCC

22201 CGTCATCGAA ACCGTGTACC TGCGCACGCC CTTCTCGGCC GGCAACGCCA  
GCAGTAGCTT TGGCACATGG ACGCGTGCGG GAAGAGCCGG CCGTTGCGGT

22251 CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA  
GTTGTATTTC TTCGTTTCGT TAGATTGTTG TCGACGGCGG TACCCGAGGT

22301 GTGAGCAGGA ACTGAAAGCC ATTGTCAAAG ATCTTGTTG TGGGCCATAT  
CACTCGTCCT TGA CTTCGG TAACAGTTT TAGAACCAAC ACCCGGTATA

22351 TTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTTT CTCCACACAA  
AAAAACCCGT GGATACTGTT CGCGAAAGGT CCGAAACAAA GAGGTGTGTT

22401 GCTCGCCTGC GCCATAGTCA ATACGGCCGG TCGCGAGACT GGGGGCGTAC  
CGAGCGGACG CCGTATCAGT TATGCCGGCC AGCGCTCTGA CCCCCGATG

22451 ACTGGATGGC CTTTGCCTGG AACCCGCACT CAAAAACATG CTACCTCTTT  
TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTGTAC GATGGAGAAA

22501 GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA  
CTCGGGAAAC CGAAAAGACT GGTCGCTGAG TTCGTCCAA TGGTCAAAC

22551 GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCCGACCGCT  
CATGCTCAGT GAGGACGCGG CATCGCGGTA ACGAAGAAGG GGGCTGGCGA

22601 GTATAACGCT GGAAAAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC  
CATATTGCGA CCTTTTCAGG TGGGTTTCGC ATGTCCCCGG GTTGAGCCGG

22651 GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCTTTG CCAACTGGCC  
CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTGACCGG

22701 CCAAACCTCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC  
GGTTTGAGGG TACCTAGTGT TGGGGTGTA CTTGGAATAA TGGCCCCATG

Figure 26 x

22801 CAGGAACAGC TCTACAGCTT CCTGGAGCGC CACTCGCCCT ACTTCGCGAG  
GTCCTTGTCG AGATGTCGAA GGACCTCGCG GTGAGCGGGA TGAAGGCGTC

22851 CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTGTGAC TTGAAAAACA  
GGTGTACGCG GTCTAATCCT CGCGGTGAAG AAAACAGTG AACTTTTGT

22901 TGTAATAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT  
ACATTTTAT TACATGATCT CTGTGAAAGT TATTTCGTT TACGAAATA

22951 TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT  
AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGCG AGACGCGGCA

23001 TTAAAAATCA AAGGGGTTCT GCCGCGCATC GCTATGCGCC ACTGGCAGGG  
AATTTTTAGT TTCCCAAGA CGGCGCGTAG CGATACGCGG TGACCGTCCC

23051 ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAACTC AGGCACAACC  
TGTGCAACGC TATGACCACA AATCACGAGG TGAATTTGAG TCCGTGTTGG

23101 ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC  
TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGGTAGTG

23151 CAACGCGTTT AGCAGGTCGG GCGCCGATAT CTTGAAGTCG CAGTTGGGGC  
GTTGCGCAA TCCTCCAGCC CGCGGCTATA GAACTTCAGC GTCAACCCCG

23201 CTCCGCCCTG CGCGCGCGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC  
GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGACCTTG

23251 ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT GTTCGGAGAT  
TGATAGTCGC GGCCCAACCAC GTGCGACCGG TCGTGCGAGA ACAGCCTCTA

23301 CAGATCCGCG TCCAGGTCCT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT  
GTCTAGGCGC AGGTCCAGGA GCGCAACGA GTCCCGCTTG CCTCAGTTGA

23351 TTGGTAGCTG CCTTCCCAA AAGGGCGCGT GCCCAGGCTT TGAGTTGCAC  
AACCATCGAC GGAAGGGTTT TTCCCGCGCA CGGGTCCGAA ACTCAACGTG

23401 TCGCACCGTA GTGGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG  
AGCGTGGCAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGCAATCC

23451 ATACAGCGCC TGCATAAAG CCTTGATCTG CTTAAAGCC ACCTGAGCCT  
TATGTCGCGG ACGTATTTTC GGAAGTAGAC GAATTTTCGG TGGACTCGGA

23501 TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AAAGTGATTG  
AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTTGACTAAC

23551 GCCGGACAGG CCGCGTCGTG CACGCAGCAC CTTGCGTCGG TGTTGGAGAT  
CGGCCTGTCC GGCGCAGCAC GTGCGTCGTG GAACGCAGCC ACAACCTCTA

23601 CTGCAACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCTTGCTAG  
GACGTGGTGT AAAGCCGGGG TGGCCAAGAA GTGCTAGAAC CGGAACGATC

23651 ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTTCA  
TGACGAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGGTAAAGT

Figure 26Y

23701 ATCACGTGCT CCTTATTTAT CATAATGCTT CCGTGTAGAC ACTTAAGCTC  
TAGTGCACGA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG

23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT  
CGGAAGCTAG AGTCGCGTCG CCACGTCGGT GTTGC GCGTC GGGCACCCGA

23801 CGTGATGCTT GTAGGTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG  
GCACTACGAA CATCCAGTGG AGACGTTTGC TGACGTCCAT GCGGACGTCC

23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTCAGCTG  
TTAGCGGGGT AGTAGCAGTG TTTCCAGAAC AACGACCACT TCCAGTCGAC

23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTGCATACG GCCGCCAGAG  
GTTGGGCGCC ACGAGGAGCA AGTCGGTCCA GAACGTATGC CCGCGGTCTC

23951 CTTCCACTTG GTCAGGCAGT AGTTTGAAGT TCGCCTTTAG ATCGTTATCC  
GAAGGTGAAC CAGTCCGTCA TCAAACTTCA AGCGGAAATC TAGCAATAGG

24001 ACGTGGTACT TGTCCATCAG CGCGCGCGCA GCCTCCATGC CCTTCTCCCA  
TGCACCATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT

24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCGTA ATTTCACTTT  
GCGTCTGTGC TAGCCGTGTG AGTCGCCCCA GTAGTGGCAT TAAAGTGAAA

24101 CCGCTTCGCT GGGCTCTTCC TCTTCCTCTT GCGTCCGCAT ACCACGCGCC  
GGCGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGCGCGG

24151 ACTGGGTCGT CTTCAATCAG CCGCCGCACT GTGCGCTTAC CTCCTTTGCC  
TGACCCAGCA GAAGTAAGTC GGCGGCGTGA CACGCGAATG GAGGAAACGG

24201 ATGCTTGATT AGCACCGGTG GGTGCTGAA ACCCACCATT TGTAGCGCCA  
TACGAACATA TCGTGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT

24251 CATCTTCTCT TTCTTCCTCG CTGTCCACGA TTACCTCTGG TGATGGCGGG  
GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC

24301 CGCTCGGGCT TGGGAGAAGG GCGCTTCTTT TTCTTCTTGG GCGCAATGGC  
GCGAGCCCGA ACCCTCTTCC CGCGAAGAAA AAGAAGAACC CGCGTTACCG

24351 CAAATCCGCC GCCGAGGTCG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA  
GTTTAGGCGG CGGCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT

24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCTT CGGACTCGAT ACGCCGCCTC  
CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TGCGGCGGAG

24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGGC GCGGCGGACG GGGACGGGGA  
TAGGCGAAAA AACCCCGCGG GGCCCCCTCC CCGCCGCTGC CCCTGCCCCT

24501 CGACACGTCC TCCATGGTTG GGGGACGTCG CGCCGCACCG CGTCCGCGCT  
GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGGC GCAGGCGCGA

24551 CGGGGGTGGT TTCGCGCTGC TCCTCTTCCC GACTGGCCAT TTCCTTCTCC  
GCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG

24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC  
ATATCCGTCT TTTTCTAGTA CCTCAGTCAG CTCTTCTTCC TGTCGGATTG

24701 CTACCACCTT CCCCCTCGAG GCACCCCGC TTGAGGAGGA GGAAGTGATT  
GATGGTGGAA GGGGCAGCTC CGTGGGGGCG AACTCCTCTT CTTTCACTAA

24751 ATCGAGCAGG ACCCAGGTTT TGTAAGCGAA GACGACGAGG ACCGCTCAGT  
TAGCTCGTCC TGGGTCCAAA ACATTGCTT CTGCTGCTCC TGGCGAGTCA

24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG  
TGGTTGTCTC CTATTTTTCG TTCTGGTCCT GTTGCGTCTC CGTTTGCTCC

24851 AACAAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA  
TTGTTTACGCC CGCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCCT

24901 GACGACGTGC TGTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA  
CTGCTGCACG ACAACTTCGT AGACGTCGCG GTCACGCGGT AATAGACGCT

24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCTT CGCCATAGCG GATGTCAGCC  
GCGCAACGTT CTCGCGTCGC TACACGGGGA GCGGTATCGC CTACAGTCGG

25001 TTGCCTACGA ACGCCACCTA TTCTCACC GC GTACCCCA CAAACGCCAA  
AACGGATGCT TGCAGTGGAT AAGAGTGGCG CGCATGGGGG GTTTGCGGTT

25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCGGTATT  
CTTTTGCCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA

25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACCTGCA  
ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAAG GTTTTGACGT

25151 AGATACCCCT ATCCTGCCGT GCCAACCAGC GCCGAGCGGA CAAGCAGCTG  
TCTATGGGGA TAGGACGGCA CGGTGGCGT CGGCTCGCCT GTTCGTCGAC

25201 GCCTTGCGGC AGGGCGCTGT CATACTGAT ATCGCCTCGC TCAACGAAGT  
CGGAACGCCG TCCCGCGACA GTATGGACTA TAGCGGAGCG AGTTGCTTCA

25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAAACG  
CGGTTTTTAG AAACCTCCAG AACCTGCGCT GCTCTTCGCG CGCCGTTTGC

25301 CTCTGCAACA GGAAAACAGC GAAAATGAAA GTCACCTCTG AGTGTTGGTG  
GAGACGTTGT CCTTTTGTCT CTTTACTTT CAGTGAGACC TCACAACCAC

25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAAC GCAGCATCGA  
CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTTG CGTCGTAGCT

25401 GGTACCCAC TTTGCCTACC CGGCACTTAA CCTACCCCCC AAGGTCATGA  
CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGG TTCCAGTACT

25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG  
CGTGTCAGTA CTCACGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC

25501 GATGCAAATT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA  
CTACGTTTAA ACGTTCTTGT TTGTCTCTC CCGGATGGGC GTCAACCGCT

25551 CGAGCAGCTA GCGCGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGGAGG  
GCTCGTCGAT CGCGCGACCG AAGTTTGCGC GCTCGGACGG CTGAACCTCC

Figure 26 AA

25651 TGCATGCAGC GGTTCCTTGC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA  
ACGTACGTCG CCAAGAAACG ACTGGGCTC TACGTCGCGT TCGATCTCCT

25701 AACATTGCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA  
TTGTAACGTG ATGTGGAAG CTGTCCCGAT GCATGCGGTC CGGACGTTCT

25751 TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGCAC  
AGAGGTTGCA CCTCGAGACG TTGGACCAGA GGATGGAACC TTAAAACGTG

25801 GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC  
CTTTTGGCGG AACCCGTTTT GCACGAAGTA AGGTGCGAGT TCCCGCTCCG

25851 GCGCCGCGAC TACGTCCGCG ACTGCGTTTA CTTATTTCTA TGCTACACCT  
CGCGGCGCTG ATGCAGGCGC TGACGCAAAT GAATAAAGAT ACGATGTGGA

25901 GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC  
CCGTCTGCCG GTACCCGCAA ACCGTCGTCA CGAACCTCCT CACGTTGGAG

25951 AAGGAGCTGC AGAAACTGCT AAAGCAAAAC TTGAAGGACC TATGGACGGC  
TTCTTCGACG TCTTTGACGA TTTCGTTTGG AACTTCCTGG ATACCTGCCG

26001 CTTCAACGAG CGCTCCGTGG CCGCGCACCT GCGGGACATC ATTTTCCCCG  
GAAGTTGCTC GCGAGGCACC GCGCGTGGA CCGCCTGTAG TAAAAGGGGC

26051 AACGCCTGCT TAAAACCTG CAACAGGGTC TGCCAGACTT CACCAGTCAA  
TTGCGGACGA ATTTTGGGAC GTTGTCCCAG ACGGTCTGAA GTGGTCAGTT

26101 AGCATGTTGC AGAACTTTAG GAACTTTATC CTAGAGCGCT CAGGAATCTT  
TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGCGA GTCCTTAGAA

26151 GCGCGCCACC TGCTGTGCAC TTCCTAGCGA CTTTGTGCCC ATTAAGTACC  
CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCATGG

26201 GCGAATGCCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC  
CGCTTACGGG AGGCGGCGAA ACCCCGGTGA CGATGGAAGA CGTCGATCGG

26251 AACTACCTTG CCTACCACTC TGACATAATG GAAGACGTGA GCGGTGACGG  
TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC

26301 TCTACTGGAG TGTCACTGTC GCTGCAACCT ATGCACCCCG CACCGCTCCC  
AGATGACCTC ACAGTGACAG CGACGTTGGA TACGTGGGGC GTGGCGAGGG

26351 TGGTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT  
ACCAAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGAAA

26401 GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTTGAA  
CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCGGAG GCCCAACTT

26451 ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAAA TTTGTACCTG  
TGAGTGAGGC CCCGACACCT GCAGCCGAAT GGAAGCGTTT AAACATGGAC

26501 AGGACTACCA CGCCCACGAG ATTAGGTTCT ACGAAGACCA ATCCCGCCCG  
TCCTGATGGT GCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC

Figure 26 AB

26551 CCTAATGCGG AGCTTACCGC CTGCGTCATT ACCCAGGGCC ACATTCTTGG  
GGATTACGCC TCGAATGGCG GACGCAGTAA TGGGTCCCGG TGTAAAGAACC

26601 CCAATTGCAA GCCATCAACA AAGCCCGCCA AGAGTTTCTG CTACGAAAGG  
GGTTAACGTT CGGTAGTTGT TTCGGGCGGT TCTCAAAGAC GATGCTTTCC

26651 GACGGGGGGT TTA CTGAGC CCCCAGTCCG GCGAGGAGCT CAACCCAATC  
CTGCCCCCA AATGAACCTG GGGGTCAGGC CGCTCCTCGA GTTGGGTTAG

26701 CCCCCGCCGC CGCAGCCCTA TCAGCAGCAG CCGCGGGCCC TTGCTTCCCA  
GGGGGCGGCG GCGTCGGGAT AGTCGTCGTC GGCGCCCGGG AACGAAGGGT

26751 GGATGGCACC CAAAAAGAAG CTGCAGCTGC CGCCGCCACC CACGGACGAG  
CCTACCGTGG GTTTTTCTTC GACGTCGACG GCGGCGGTGG GTGCCTGCTC

26801 GAGGAATACT GGGACAGTCA GGCAGAGGAG GTTTTGGACG AGGAGGAGGA  
CTCCTTATGA CCCTGTCAGT CCGTCTCCTC CAAAACCTGC TCCTCCTCCT

26851 GGACATGATG GAAGACTGGG AGAGCCTAGA CGAGGAAGCT TCCGAGSTCG  
CCTGTACTAC CTTCTGACCC TCTCGGATCT GCTCCTTCGA AGGCTCCAGC

26901 AAGAGGTGTC AGACGAAACA CCGTCACCCT CGGTGCGATT CCCCTCGCCG  
TTCTCCACAG TCTGCTTTGT GGCAGTGGGA GCCAGCGTAA GGGGAGCGGC

26951 GCGCCCCAGA AATCGGCAAC CGGTTCCAGC ATGGCTACAA CCTCCGCTCC  
CGCGGGGTCT TTAGCCGTTG GCCAAGGTCG TACCGATGTT GGAGGCGAGG

27001 TCAGGCGCCG CCGGCACTGC CCGTTCGCCG ACCCAACCGT AGATGGGACA  
AGTCCGCGGC GGCCGTGACG GGCAAGCGGC TGGGTGGCA TCTACCTGT

27051 CCACTGGAAC CAGGGCCGGT AAGTCCAAGC AGCCGCGGCC GTTAGCCCAA  
GGTGACCTTG GTCCCGGCCA TTCAGGTTCTG TCGGCGGCGG CAATCGGGTT

27101 GAGCAACAAC AGCGCCAAGG CTACCGCTCA TGGCGCGGGC ACAAGAAGCG  
CTCGTTGTTG TCGCGGTTCC GATGGCGAGT ACCGCGCCCG TGTCTTTGCG

27151 CATAGTTGCT TGCTTGCAAG ACTGTGGGGG CAACATCTCC TTCGCCCCGC  
GTATCAACGA ACGAACGTTT TGACACCCCC GTTGTAGAGG AAGCGGGCGG

27201 GCTTTCTTCT CTACCATCAC GCGGTGGCCT TCCCCGTAA CATCCTGCAT  
CGAAAGAAGA GATGGTAGTG CCGCACCGGA AGGGGGCATT GTAGGACGTA

27251 TACTACCGTC ATCTCTACAG CCCATACTGC ACCGGCGGCA GCGGCAGCAA  
ATGATGGCAG TAGAGATGTC GGGTATGACG TGGCCGCCGT CGCCGTCGTT

27301 CAGCAGCGGC CACACAGAAG CAAAGGCGAC CGGATAGCAA GACTCTGACA  
GTGCTCGCCG GTGTGTCTTC GTTCCGCTG GCCTATCGTT CTGAGACTGT

27351 AAGCCCAAGA AATCCACAGC GCGGCAGCA GCAGGAGGAG GAGCGCTGCG  
TTCGGGTCT TTAGGTGTCG CCGCCGTCGT CGTCTCCTC CTCGCGACGC

27401 TCTGGCGCCC AACGAACCCG TATCGACCCG CGAGCTTAGA AACAGGATTT  
AGACCGCGGG TTGCTTGGGC ATAGCTGGGC GCTCGAATCT TTGTCTTAAA

27451 TTCCCACTCT GTATGCTATA TTTCAACAGA GCAGGGGCCA AGAACAAGAG  
AAGGGTGAGA CATACGATAT AAAGTTGTCT CGTCCCCGGT TCTTGTCTCT

Figure 26: AC



27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC  
AGTGTTCG CTTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG

27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT  
AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA

27651 TCTCAAATTT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC  
AGAGTTTAAA TTCGCGCTTT TGATGCAGTA GAGGTCGCCG GTGTGGGCCG

27701 GCCAGCACCT GTTGTGACGG CCATTATGAG CAAGGAAATT CCCACGCCCT  
CGGTCGTGGA CAACAGTCGC GGTAATACTC GTTCCTTTAA GGGTGCGGGA

27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA  
TGTACACCTC AATGGTCGGT GTTTACCCTG AACGCCGACC TCGACGGGTT

27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC  
CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG

27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG  
GGCCCACTTG CCTTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTCC

27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCCG TAGTTGGCCC  
GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG

27951 GCTGCCCTGG TGTACCAGGA AAGTCCCGCT CCCACCACTG TGGTACTTCC  
CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG

28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG  
GTCTCTGCGG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC

28051 CGGGCGGCTT TCGTCACAGG GTGCGGTGCG CCGGGCAGGG TATAACTCAC  
GCCCCCGAA AGCAGTGTCC CACGCCAGCG GGCCCGTCCC ATATTGAGTG

28101 CTGACAATCA GAGGGCGAGG TATTCAGCTC AACGACGAGT CGGTGAGCTC  
GACTGTTAGT CTCCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG

28151 CTCGCTTGGT CTCCGTCCGG ACGGGACATT TCAGATCGGC GGCGCCGGCC  
GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCCGG

28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC  
CGAGAAGTAA GTGCGGAGCA GTCCGTTAGG ATTGAGACGT CTGGAGCAGG

28251 TCTGAGCCGC GCTCTGGAGG CATTGGAACT CTGCAATTTA TTGAGGAGTT  
AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTTAAAT AACTCCTCAA

28301 TGTGCCATCG GTCTACTTTA ACCCCTTCTC GGGACCTCCC GGCCACTATC  
ACACGGTAGC CAGATGAAAT TGGGGAAGAG CCCTGGAGGG CCGGTGATAG

28351 CGGATCAATT TATTCCTAAC TTTGACGCGG TAAAGGACTC GGCGGACGGC  
GCCTAGTTAA ATAAGGATTG AAAGTGCGCC ATTTCTGAG CCGCCTGCCG

28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT  
ATGCTGACTT ACAATTACCC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

28451 GGTCCACTGT CGCCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTTT  
CCAGGTGACA GCGGCGGTGT TCACGAAACG GCGCTGAGG CCACTCAAAA

28501 GCTACTTTGA ATTGCCCCGAG GATCATATCG AGGGCCCCGC GCACGGCGTC  
CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGAG

28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTC GGGAGTTTAC  
GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG

28601 CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG  
GGTCGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC

28651 TGATTGCAA CTGTCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT  
ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA

28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAATATAC TGGGGCTCCT  
GAGACACGAC TCATATTATT TATGTCTTTA ATTTATATG ACCCCGAGGA

28751 ATCGCCATCC TGTAACGCC ACCGTCTTCA CCCGCCAAG CAAACCAAGG  
TAGCGGTAGG ACATTGCGG TGGCAGAAGT GGGCGGGTTC GTTGGTTCC

28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG  
GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC

28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT  
AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA

28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGA ACGTACGAGT  
TGAGGTAGTC TTTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA

28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT  
CGCAGTGGCC GCGGACGTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA

29001 TTTCGGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT  
AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTTGTC TCCACTCGAA

29051 AGAAAACCCT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT  
TCTTTTGGGA ATCCATAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA

29101 GAACAATTCA AGCAACTCTA CGGGCTATTC TAATTCAGGT TTCTCTAGAA  
CTTGTTAAGT TCGTTGAGAT GCCGATAAG ATTAAGTCCA AAGAGATCTT

29151 TCGGGGTTGG GGTATTCTC TGTCTTGTA TTCTCTTTAT TCTTATACTA  
AGCCCCAACC CCAATAAGAG ACAGAACACT AAGAGAAATA AGAATATGAT

29201 ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA  
TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT

29251 TTGTCAGCTT TTTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT  
AACAGTCGAA AAATTTGCGA CCCAGCGGT GGGTTCTACT AATCCATGTA

29301 AATCCTAGGT TTACTACCCC TTGCGTCAGC CCACGGTACC ACCCAAAGG  
TTAGGATCCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTTCC

29351 TGGATTTTAA GGAGCCAGCC TGTAATGTTA CATTGCGAGC TGAAGCTAAT  
ACCTAAAATT CCTCGGTGCG ACATTACAAT GTAAGCGTCG ACTTCGATTA

Figure 26 AE

29451 TCGCCACAAA AACAAAATTG GCAAGTATGC TGTTTATGCT ATTTGGCAGC  
AGCGGTGTTT TTGTTTAAAC CGTTCATACG ACAAATACGA TAAACCGTCG

29501 CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT  
GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA

29551 AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT  
TTTTGAAAAT ACATATGAAA AGGTAAAATA CTTTACACGC TGTAATGGTA

29601 GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGAAA  
CATGTACTCG TTTGTCTAT TCAACACCGG GGGTGTTTTA ACACACCTTT

29651 ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTTG  
TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC

29701 GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTATTGA  
CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT

29751 GGAAAAGAAA ATGCCTTAAT TTACTAAGTT ACAAAGCTAA TGTCACCACT  
CCTTTTCTTT TACGGAATTA AATGATTCAA TGTTTCGATT ACAGTGGTGA

29801 AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA  
TTGACGAAAT GAGCGACGAA CGTTTGTGTT AAGTTTTTCA ATCGTAATAT

29851 ATTAGAATAG GATTTAAACC CCCCAGTCAT TTCCTGCTCA ATACCATTCC  
TAATCTTATC CTAAATTTGG GGGGCCAGTA AAGGACGAGT TATGGTAAGG

29901 CCTGAACAAT TGACTCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA  
GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTCGCGAT GTTGGAACCT

29951 GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC  
CAGTCCGAAG GACCTACAGT CGTAGACTGA AACCGGTCGT GGACAGGGCG

30001 GGATTTGTTT CAGTCCAAC ACAGCGACCC ACCCTAACAG AGATGACCAA  
CCTAAACAAG GTCAGGTTGA TGTGCTGGG TGGGATTGTC TCTACTGGTT

30051 CACAACCAAC GCGGCCGCCG CTACCGGACT TACATCTACC ACAAATACAC  
GTGTTGGTTG CGCCGGCGGC GATGGCCTGA ATGTAGATGG TGTATTATGTG

30101 CCCAAGTTTC TGCCTTTGTC AATAACTGGG ATAACCTGGG CATGTGGTGG  
GGGTTCAAAG ACGGAAACAG TTATTGACCC TATTGAACCC GTACACCACC

30151 TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG  
AAGAGGTATC GCGAATACAA ACATACGGAA TAATAATACA CCGAGTAGAC

30201 CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG  
GACGGATTTT GCGTTTGCGC GGGCTGGTGG GTAGATATCA GGGTAGTAAC

30251 TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC  
ACGATGTGGG TTTGTTACTA CCTTAGGTAT CTAACCTGCC TGACTTTGTG

30301 ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT  
TACAAGAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA

Figure 26 AF

30401 TGCGGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT  
ACGCCAAAGA GTGTAGCTTC ATCTGACGTA AGGTCGGAAG TGTCAGATAA

30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG  
ACGAAATGCC TAAACAGTGG GAGTGCGAGT AGACGTCGGA GTAGTGACAC

30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA  
CAGTAGCGGA AATAGGTCAC GTAACGTACC CAGACACACG CGAAACGTAT

30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA  
AGAGTCTGTG GTAGGGGTCA TGTCCTGTC CTGATATCGA CTCGAAGAAT

30601 GAATTCTTTA ATTATGAAAT TTAATGTGAC TTTTCTGCTG ATTATTTGCA  
CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAAACGT

30651 CCCTATCTGC GTTTTGTTC CCGACCTCCA AGCCTCAAAG ACATATATCA  
GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT

30701 TGCAGATTCA CTCGTATATG GAATATTCCA AGTTGCTACA ATGAAAAAAG  
ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTTC

30751 CGATCTTTCC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTCTT  
GCTAGAAAGG CTTCCGACCA ATATACGTTA GTAGAGACAA TACCACAAGA

30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTGGCTGG  
CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAAC GTAAACCGACC

30851 AACGCAATAG ATGCCATGAA CCACCCAACT TTCCCCGCGC CCGCTATGCT  
TTGCGTTATC TACGGTACTT GGTGGGTTGA AAGGGGCGCG GCGGATACGA

30901 TCCACTGCAA CAAGTTGTTG CCGGCGGCTT TGTCCCAGCC AATCAGCCTC  
AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGGTCGG TTAGTCGGAG

30951 GCCCACCTTC TCCCACCCCT ACTGAAATCA GCTACTTTAA TCTAACAGGA  
CGGGTGAAG AGGGTGGGGG TGACTTTAGT CGATGAAATT AGATTGTCCT

31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC  
CCTCTACTGA CTGTGGGATC TAGATCTTTA CCTGCCTTAA TAATGTCTCG

31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCGG CCGAGCAACA GCGCATGAAT  
TCGCGGACGA TCTTTCTGCG TCCCGTCGCC GGCTCGTTGT CCGGTACTTA

31101 CAAGAGCTCC AAGACATGGT TAACCTGCAC CAGTGCAAAA GGGGTATCTT  
GTTCTCGAGG TTCTGTACCA ATTGAACGTG GTCACGTTT CCCCATAGAA

31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC  
AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCATTA TGGTGGCCTG

31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG  
TGGCGGAATC GATGTTCAAC GGTGGGTTTC CAGTCTTTAA CCACCAGTAC

31251 GTGGGAGAAA AGCCCATAC CATAACTCAG CACTCGGTAG AAACCGAAGG  
CACCCTCTTT TCGGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 24 AG

31351 AGACCCTGTG CGGTCTCAAA GATCTTATTC CCTTTAACTA ATAAAAAAAA  
TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATTGAT TATTTTTTTT

31401 ATAATAAAGC ATCACTTACT TAAATCAGT TAGCAAATTT CTGTCCAGTT  
TATTATTTTCG TAGTGAATGA ATTTTAGTCA ATCGTTTAA GACAGGTCAA

31451 TATTCAGCAG CACCTCCTTG CCTCCTCCC AGCTCTGGTA TTGCAGCTTC  
ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG

31501 CTCCTGGCTG CAACTTTCT CCACAATCTA AATGGAATGT CAGTTTCCTC  
GAGGACCGAC GTTGAAGA GGTGTTAGAT TTACCTTACA GTCAAAGGAG

31551 CTGTTCTGT CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC  
GACAAGGACA GGTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG

31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGATACC ATATGACAGC  
CGCGTTCTGG CAGACTTCTA TGGAAGTTGG GGCACATAGG TATACTGTGC

31651 GAAACCGGTC CTCCAACGT GCCTTTTCTT ACTCCTCCCT TTGTATCCCC  
CTTTGGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG

31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG  
GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC

31751 AACCTCTAGT TACCTCCAAT GGCATGCTTG CGCTCAAAAT GGGCAACGGC  
TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTAA CCCGTTGCCG

31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAATG TAACCACTGT  
GAGAGAGACC TGCTCCGGCC GTTGAATGG AGGGTTTTAC ATTGGTGACA

31851 GAGCCCACCT CTCAAAAAA CCAAGTCAAA CATAAACCTG GAAATATCTG  
CTCGGGTGGA GAGTTTTTTT GGTTCAGTTT GTATTTGGAC CTTTATAGAC

31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT  
GTGGGGAGTG TCAATGGAGT CTTGCGGATT GACACCGACG GCGGCGTGGA

31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCCTAAC  
GATTACCAGC GCGGTTGTG TGAGTGGTAC GTTAGTGTC GGGGCGATTG

32001 CGTGCACGAC TCCAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT  
GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTCA

32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT  
GTCTTCCTTT CGATCGGGAC GTTGTAGTC CGGGGAGTG GTGGTGGCTA

32101 AGCAGTACCC TTAATATCAC TGCCTCACC CCTCTAACTA CTGCCACTGG  
TCGTATGGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC

32151 TAGCTTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAC  
ATCGAACCCG TAACTGAAC TTCTCGGGTA AATATGTGTT TTACCTTTTG

32201 TAGGACTAAA GTACGGGGCT CCTTTGCATG TAACAGACGA CCTAAACACT  
ATCCTGATTT CATGCCCGA GGAAACGTAC ATTGTCTGCT GGATTTGTGA

Figure 26 AH

32301 AACTAAAGTT ACTGGAGCCT TGGGTTTTGA TTCACAAGGC AATATGCAAC  
TTGATTTCAA TGACCTCGGA ACCCAAACT AAGTGTTCG TTATACGTTG

32351 TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA  
AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTTGTC TGCGGAATAT

32401 CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCAACTAA ATCTAAGACT  
GAACTACAAT CAATAGGCAA ACTACGAGTT TTGGTTGATT TAGATTCTGA

32451 AGGACAGGGC CCTCTTTTTA TAAACTCAGC CCACAACCTG GATATTAAC  
TCCTGTCCCG GGAGAAAAAT ATTTGAGTCG GGTGTTGAAC CTATAATTGA

32501 ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAAGCTT  
TGTTGTTTCC GGAAATGAAC AAATGTCGAA GTTGTTAAG GTTTTTCGAA

32551 GAGGTTAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT  
CTCCAATTGG ATTCGTGACG GTTCCCCAAC TACAACTGC GATGTCGGTA

32601 AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA  
TCGGTAATTA CGTCCTCTAC CCGAACTTAA ACCAAGTGA TTACGTGGTT

32651 ACACAAATCC CCTCAAAACA AAAATTGGCC ATGGCCTAGA ATTTGATTCA  
TGTGTTTAGG GGAGTTTGT TTTTAACCGG TACCGGATCT TAAACTAAGT

32701 AACAAAGGCTA TGGTTCCTAA ACTAGGAACT GGCCTTAGTT TTGACAGCAC  
TTGTTCCGAT ACCAAGGATT TGATCCTGA CCGGAATCAA AACTGTCTGT

32751 AGGTGCCATT ACAGTAGGAA AAAAAATAA TGATAAGCTA ACTTGTGGA  
TCCACGGTAA TGTCATCCTT TGTTTTTATT ACTATTCGAT TGAAACACCT

32801 CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT  
GGTGTGGTCG AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA

32851 AAACCTCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT  
TTTGAGTGAA ACCAGAATTG TTTTACACCG TCAGTTTATG AACGATGTCA

32901 TTCAGTTTTG GCTGTAAAG GCAGTTTGGC TCCAATATCT GGAACAGTTC  
AAGTCAAAAC CGACAATTTC CGTCAAACCG AGGTTATAGA CTTGTCAAG

32951 AAAGTGCTCA TCTTATTATA AGATTTGACG AAAATGGAGT GCTACTAAAC  
TTTCACGAGT AGAATAATAT TCTAACTGC TTTTACCTCA CGATGATTG

33001 AATTCCTTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC  
TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTTAC CTCTAGAATG

33051 TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG  
ACTTCCGTGT CGGATATGTT TGCGACAACC TAAATACGGA TTGGATAGTC

33101 CTTATCCAAA ATCTACGGT AAAACTGCCA AAAGTAACAT TGTCAGTCAA  
GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTCATTGTA ACAGTCAGTT

33151 GTTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT  
CAAATGAATT TGCCTCTGTT TTGATTGGA CATTGTGATT GGTAATGTGA

Figure 26 AI

33251 CATTTCATG GGA CTGGTCT GGCCACA ACT ACATTAATGA AATATTTGCC  
 GTAAAAGTAC CCTGACCAGA CCGGTGTTGA TGTAACTACT TTATAAACGG

33301 ACATCCTCTT ACACCTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG  
 TGTAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAAC

33351 TGTATGTTT CAACGTGTTT ATTTTCAAT TGCAGAAAAT TTCAAGTCAT  
 ACAATACAAA GTTGACACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA

33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC  
 AAAAGTAAGT CATCATATCG GGGTGGTGGT GTATCGAATA TGTCTAGTGG

33451 GTACCTTAAT CAAACTCACA GAACCCTAGT ATTCAACCTG CCACCTCCCT  
 CATGGAATTA GTTTGAGTGT CTTGGGATCA TAAGTTGGAC GGTGGAGGGA

33501 CCCAACACAC AGAGTACACA GTCCTTCTC CCCGGCTGGC CTTAAAAAGC  
 GGGTTGTGTG TCTCATGTGT CAGGAAAGAG GGGCCGACCG GAATTTTTTCG

33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT  
 TAGTATAGTA CCCATTGTCT GTATAAGAAT CCACAATATA AGGTGTGCCA

33601 TTCCTGTCGA GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA  
 AAGGACAGCT CGGTTTGC GA TAGTCACTA TAATTATTG AGGGGCCCCGT

33651 GCTCACTTAA GTTCATGTG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT  
 CGAGTGAATT CAAGTACAGC GACAGGTCGA CGACTCGGTG TCCGACGACA

33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT  
 GGTGGAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TGCGGATGTA

33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA  
 CCCCCATCTC AGTATTAGCA CGTAGTCTTA TCCCGCCACC ACGACGTCGT

33801 GCGCGCGAAT AACTGCTGC CGCCGCCGCT CCGTCCTGCA GGAATACAAC  
 CGCGCGCTTA TTTGACGACG GCGGCGGCGA GGCAGGACGT CCTTATGTTG

33851 ATGGCAGTGG TCTCCTCAGC GATGATTCGC ACCGCCC GCA GCATAAGGCG  
 TACCGTCACC AGAGGAGTCG CTACTAAGCG TGGCGGGCGT CGTATTCCGC

33901 CCTTGTCTC CGGGCACAGC AGCGCACCTT GATCTCACTT AAATCAGCAC  
 GGAACAGGAG GCCCGTGTG TCGCGTGGGA CTAGAGTGAA TTTAGTCGTG

33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG  
 TCATTGACGT CGTGTCGTGG TGTATAACA AGTTTTAGGG TGTCACGTTT

34001 GCGCTGTATC CAAAGCTCAT GCGGGGACC ACAGAACCCA CGTGGCCATC  
 CGCGACATAG GTTTCGAGTA CCGCCCCTGG TGTCTTGGGT GCACCGGTAG

34051 ATACCACAAG CGCAGGTAGA TTAAGTGCG ACCCCTCATA AACACGCTGG  
 TATGGTGTTC GCGTCCATCT AATTCACCGC TGGGGAGTAT TTGTGCGACC

34101 ACATAAACAT TACCTCTTTT GGCATGTTGT AATTCACCAC CTCCCGGTAC  
 TGTATTTGTA ATGGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34201 GCTGGCCAAA ACCTGCCCCG CGGCTATACA CTGCAGGGAA CCGGGACTGG  
CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC

34251 AACAATGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC  
TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG

34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT  
CAGTACTATA GTTACAACCG TGTGTGTGCC GTGTGCACGT ATGTGAAGGA

34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC  
GTCCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTTGTGGG

34401 ATTCTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA  
TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT

34451 CTCACGTTGT GCATTGTCAA AGTGTTACAT TCGGGCAGCA GCGGATGATC  
GAGTGCAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG

34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC  
GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTCTCTCCA TCTGCTAGGG

34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTTGG TCGTAGTGTG  
ATGACATGCC TCACGCGGCT CTGTTGGCTC TAGCACAACC AGCATCACAG

34601 ATGCCAAATG GAACGCCGGA CGTAGTCATA TTTCTGAAG CAAAACCAGG  
TACGGTTTAC CTTGCGGCCT GCATCAGTAT AAAGGACTTC GTTTTGGTCC

34651 TGCGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC  
ACGCCCCGAC TGTTTGTCTA GACGCAGAGG CCAGAGCGGC GAATCTAGCG

34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC  
AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCCGCGGG

34751 CCTGGCTTCG GGTTCTATGT AAACCTCTTC ATGCGCGGCT GCCCTGATAA  
GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGCGCA CGGGACTATT

34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTCTGTT  
GTAGGTGGTG GCGTCTTATT CGGTGTGGGT CGGTTGGATG TGTAAGCAAG

34851 TGCGAGTCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT  
ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA

34901 TTTTTTATTC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAAG  
AAAAAATAAG GTTTTCTAAT AGGTTTTGGA GTTTTACTTC TAGATAATTC

34951 TGAACGCGCT CCCCTCCGGT GCGTGGTCA AACTCTACAG CCAAAGAACA  
ACTTGCGCGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTTCTTGT

35001 GATAATGGCA TTTGTAAGAT GTTGCACAAT GGCTTCCAAA AGGCAAACGG  
CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCCGTTTGCC

35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC  
GGGAGTGCAG GTTCACCTGC ATTTCCGATT TGGGAAGTCC CACTTAGAGG

Figure 26AK



35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCGAATATTA AGTCCGGCCA  
GGTGGAAGAG TTATATAGAG ATTCGTTTAG GGCTTATAAT TCAGGCCGGT

35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CTTTCAGCCT CAAGCAGCGA  
AACATTTTTA GACGAGGTCT CGCGGGAGGT GGAAGTCGGA GTTCGTCGCT

35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA  
TAGTACTAAC GTTTTTAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT

35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGCAGGGC  
TCGCCTTGTA ATTGTTTTTA TGGCGCTAGG GCATCCAGGG AAGCGTCCCG

35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC  
GTCGACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG

35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC  
GCGGTCCTTG GTACTGTTTT CTTGGGTGTG ACTAATACTG TCGGTATGAG

35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG  
CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCTGAACAA CGTACCCGCC

35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA  
GCTATATTTT ACGTTCCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT

35551 AAAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAAG GCAGGTAAGC  
TTTTTCTTTC GTGTAGCATC AGTACGAGTA CGTCTATTTC CGTCCATTGC

35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC  
AGGCCTTGGT GGTGTCTTTT TCTGTGGTAA AAAGAGAGTT TGTACAGACG

35651 GGGTTTCTGC ATAAACACAA AATAAAATAA CAAAAAACA TTTAAACATT  
CCCAAAGACG TATTTGTGTT TTATTTTATT GTTTTTTTGT AAATTGTAA

35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA  
TCTTCGGACA GAATGTTGTC CTTTTGTGTT GGAATATTCTG TATTCTGCCT

35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA  
GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCAGTGG CACTAATTTT

35801 AGCACCACCG ACAGCTCCTC GGTATGTGCC GGAGTCATAA TGTAAGACTC  
TCGTGGTGGC TGTCGAGGAG CCAGTACAGG CCTCAGTATT ACATTCTGAG

35851 GGTAAACACA TCAGGTTGAT TCACATCGGT CAGTGCTAAA AAGCGACCGA  
CCATTTGTGT AGTCCAATA AGTGTAGCCA GTCACGATTT TTCGCTGGCT

35901 AATAGCCCGG GGAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC  
TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG

35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC  
GGGTATCCTC CATATTGTTT TAATTATCCT CTCTTTTTGT GTATTGTGG

36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA  
ACTTTTTGGG AGGACGGATC CGTTTTATCG TGGGAGGGCG AGGTCTTGTT

Figure 26 AL

36101 AAAGAAAACC TATTAAAAA ACACCACTCG ACACGGCACC AGCTCAATCA  
 TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGCGCCGTGG TCGAGTTAGT  
 36151 GTCACAGTGT AAAAAAGGGC CAAGTGCAGA GCGAGTATAT ATAGGACTAA  
 CAGTGTACA TTTTTCCTCG GTTCACGTCT CGCTCATATA TATCCTGATT  
 36201 AAAATGACGT AACGGTTAAA GTCCACAAAA AACACCCAGA AAACCGCACG  
 TTTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTGGCGTG  
 36251 CGAACCTACG CCCAGAAACG AAAGCCAAAA AACCCACAAC TTCCTCAAAT  
 GCTTGGATGC GGGTCTTTGC TTTTCGGTTTT TTGGGTGTTG AAGGAGTTTA  
 36301 CGTCACTTCC GTTTTCCAC GTTACGTAC TTCCCATTTT AAGAAAATA  
 GCAGTGAAG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT  
 36351 CAATTCCCAA CACATACAAG TTAATCCGCC CTAAACCTA CGTACCCGCG  
 GTTAAGGGTT GTGTATGTT AATGAGGCGG GATTTTGGAT GCAGTGGGCG  
 36401 CCCGTTCCCA CGCCCCGCGC CACGTCACAA ACTCCACCCC CTCATTATCA  
 GGGCAAGGGT GCGGGGCGCG GTGCAGTGT TGAGGTGGGG GAGTAATAGT  
 PacI  
 -----  
 36451 TATTGGCTTC AATCCAAAAT AAGGTATATT ATTGATGATG TTAATTAAGA  
 ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACTACTAC AATTAATTCT  
 36501 ATTCGGATCT GCGACGCGAG GCTGGATGGC CTTCCCCATT ATGATTCTTC  
 TAAGCCTAGA CGCTGCGCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG  
 36551 TCGCTTCCGG CGGCATCGGG ATGCCCCGCGT TGCAGGCCAT GCTGTCCAGG  
 AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGGTA CGACAGGTCC  
 36601 CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG  
 GTCCATCTAC TGCTGGTAGT CCCTGTGCGA GTTCCGGTCG TTTTCCGGTC  
 36651 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC  
 CTTGGCATT TTTCCGGCGA ACGACCGCAA AAAGGTATCC GAGGCGGGGG  
 36701 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG  
 GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC  
 36751 ACAGGACTAT AAAGATACCA GCGGTTTCCC CCTGGAAGCT CCCTCGTGCG  
 TGTCCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGG  
 36801 CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC  
 GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG  
 36851 CTTCCGGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT  
 GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA  
 36901 TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT  
 AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA

Figure 26 AM

37001 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT  
GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA

37051 AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC  
TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAAGT TCACCACCGG

37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA  
ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT

37151 AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA  
TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAAGTAG GCCGTTTGT

37201 ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG  
TGGTGGCGAC CATCGCCACC AAAAAACAA ACGTTCGTCG TCTAATGCGC

37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG  
GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC

37301 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA  
TGCGAGTCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT

37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT  
AGTTTTTCCT AGAAGTGGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA

37401 GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT  
CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA

37451 CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCCCCGTG  
GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC

37501 TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA  
ACATCTATTG ATGCTATGCC CTCCCGAATG GTAGACCGGG GTCACGACGT

37551 ATGATAACCG GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA  
TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT

37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG  
GGTCGGTCGG CCTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAAATAGGC

37651 CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG  
GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC

37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT  
GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACCA

37751 GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT  
CAGTGCGAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGGTTGCTA

37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC GGTTAGCTCC  
GTTCCGCTCA ATGTACTAGG GGGTACAACA CGTTTTTTTCG CCAATCGAGG

37851 TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCAG TGTTATCACT  
AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACC GGCGTC ACAATAGTGA

Figure 26 AN

37951 GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG  
 CTACGAAAAG ACACTGACCA CTCATGAGTT GGTTCAGTAA GACTCTTATC  
  
 38001 TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC  
 ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG  
  
 38051 CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT  
 GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA  
  
 38101 CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTGAGATC CAGTTCGATG  
 GCCCCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC  
  
 38151 TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG  
 ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGGTC  
  
 38201 CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA  
 GCAAAGACCC ACTCGTTTTT GTCCTTCCGT TTTACGGCGT TTTTCCCTT  
  
 38251 TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT  
 ATTCCCGCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA  
  
 38301 TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA  
 ATAACCTTCG AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT  
  
 38351 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA  
 TACATAAATC TTTTATTTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT  
  
 38401 AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT  
 TTCACGGTGG ACTGCAGATT CTTTGGTAAT AATAGTACTG TAATTGGATA  
  
 38451 AAAAATAGGC GTATCACGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA  
 TTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

PacI

38501 TTCTTAATTT CTTAATTAA (SEQ ID NO:32)  
 AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 A0

1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG  
GTAGTAGTTA TTATATGGAA TAAACCTAA CTTCCGGTAT ACTATTACTC

51 GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG  
CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA  
ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG  
CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTGGG  
CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAACTG AATAAGAGGA  
GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA  
TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT  
CCCCGGCGCC CTGAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG  
GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCACAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT  
CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC  
ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA  
ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG  
ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCAT GACGTCAATA ATGACGTATG TTCCCATAGT  
GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT  
TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAATGCCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC  
TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCAGTA  
GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT

Figure 27A

901 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA  
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA  
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA  
ACCCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG  
TGTGAGGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG  
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC  
GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTCGG

1201 TCCGCGGGCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT  
AGGCGCCGGC CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCCGCT  
CTCTAGACGG TGGTACCGGC CGTTCACCAG GTTCTCCAGG CACGGGCCGA

1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG  
CCAGGTGGCA CTCCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCCAGGGA  
CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG  
GGACCTCTTC GTGCCGCGGT AGTGGAGGAG GTTGTGGCGG CGGTGGTTGC

1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC  
GGCTGACGCG GACCGACCTC CGGGTCTCTC TGCTCTCCA CCCGAAGGGG

1501 GTGAGGCCCC AGGTGCCCCCT GAGGCCCCATG ACCTACAAGG GCGCCGTGGA  
CACTCCGGGG TCCACGGGGA CTCCGGGTAC TGGATGTTCC CGCGGCACCT

1551 CCTGTCCCAC TTCCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT  
GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGTACCA CACCCAGGGC  
GGGTCTTCTC CGTCCTGTAG GACCTGGACA CCCACATGGT GTGGGTCCCG

1651 TACTTCCCCG ACTGGCAGAA CTACACCCCC GGCCCCGGCA TCAGGTTCCC  
ATGAAGGGGC TGACCGTCTT GATGTGGGGG CCGGGGCCGT AGTCCAAGGG

1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCCTGGAG CCCGAGAAGG  
GGACTGGAAG CCGACCACGA AGTTCGACCA CGGGCACCTC GGGCTCTTCC

1751 TGGAGGAGGC CAACGAGGGC GAGAACAAC TCGCCGCCCA CCCCATGTCC  
ACCTCCTCCG GTTGCTCCCG CTCTTGTTGA CGCGCGGGT GGGGTACAGG

Figure 27B

1851 CTCCAAGCTG GCCTTCCACC ACGTGGCCAG GGAGCTGCAC CCCGAGTACT  
GAGGTTCGAC CGGAAGGTGG TGCACCGGTC CCTCGACGTG GGGCTCATGA

1901 ACAAGGACTG CTAAAGCCCG GGCAGATCTG CTGTGCCTTC TAGTTGCCAG  
TGTTCTCTGAC GATTTCGGGC CCGTCTAGAC GACACGGAAG ATCAACGGTC

1951 CCATCTGTTG TTTGCCCCTC CCCCCTGCCT TCCTTGACCC TGGAAGGTGC  
GGTAGACAAC AAACGGGGAG GGGGCACGGA AGGAACTGGG ACCTTCCACG

2001 CACTCCCACT GTCCTTTCCT AATAAAATGA GGAAATTGCA TCGCATTGTC  
GTGAGGGTGA CAGGAAAGGA TTATTTTACT CCTTTAACGT AGCGTAACAG

2051 TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG  
ACTCATCCAC AGTAAGATAA GACCCCCAC CCCACCCCGT CCTGTCGTTT

2101 GGGGAGGATT GGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC  
CCCCTCCTAA CCCTTCTGTT ATCGTCCGTA CGACCCCTAC GCCACCCGAG

2151 TATGGCCGAT CGGCGCGCCG TACTGAAATG TGTGGGCGTG GCTTAAGGGT  
ATACCGGCTA GCCGCGCGGC ATGACTTTAC ACACCCGCAC CGAATTCCCA

2201 GGGAAAGAAT ATATAAGGTG GGGGTCTTAT GTAGTTTGTG ATCTGTTTTG  
CCCTTTCTTA TATATTCCAC CCCCAGAATA CATCAAAACA TAGACAAAAC

2251 CAGCAGCCGC CGCCGCCATG AGCACCAACT CGTTTGATGG AAGCATTGTG  
GTCGTCGGCG GCGCGGGTAC TCGTGGTTGA GCAAACTACC TTCGTAACAC

2301 A3CTCATATT TGACAACGCG CATGCCCCCA TGGGCCGGGG TCGTCAGAA  
TCGAGTATAA ACTGTTGCGC GTACGGGGGT ACCCGGCCCC ACGCAGTCTT

2351 TGTGATGGGC TCCAGCATTG ATGGTCGCCC CGTCCTGCCC GCAAACTCTA  
ACACTACCCG AGGTCGTAAC TACCAGCGGG GCAGGACGGG CGTTTGAGAT

2401 CTACCTTGAC CTACGAGACC GTGTCTGGAA CGCCGTTGGA GACTGCAGCC  
GATGGAAGTG GATGCTCTGG CACAGACCTT GCGGCAACCT CTGACGTCGG

2451 TCCGCCGCGC CTTCAGCCGC TGCAGCCACC GCCC GCGGGA TTGTGACTGA  
AGGCGGCGGC GAAGTCGGCG ACGTCGGTGG CGGGCGCCCT AACACTGACT

2501 CTTTGCTTTC CTGAGCCCGC TTGCAAACAG TGCAGCTTCC CGTTCATCCG  
GAAACGAAAG GACTCGGGCG AACGTTTGTC ACGTCGAAGG GCAAGTAGGC

2551 CCCGCGATGA CAAGTTGACG GCTCTTTTGG CACAATTGGA TTCTTTGACC  
GGGCGCTACT GTTCAACTGC CGAGAAAACC GTGTTAACCT AAGAACTGG

2601 CGGGAACCTA ATGTCGTTTC TCAGCAGCTG TTGGATCTGC GCCAGCAGGT  
GCCCTTGAAT TACAGCAAAG AGTCGTCGAC AACCTAGACG CGGTCGTCCA

2651 TTCTGCCCTG AAGGCTTCCT CCCCTCCCAA TGCGGTTTAA AACATAAATA  
AAGACGGGAC TTCCGAAGGA GGGGAGGGTT ACGCCAAATT TTGTATTTAT

2701 AAAAACCAGA CTCTGTTTGG ATTTGGATCA AGCAAGTGTG TTGCTGTCTT  
TTTTTGGTCT GAGACAAACC TAAACCTAGT TCGTTACAG AACGACAGAA

Figure 27C

2751 TATTTAGGGG TTTTGCGCGC GCGGTAGGCC CGGGACCAGC GGTCTCGGT  
ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG

2801 GTTGAGGGTC CTGTGTATTT TTTCCAGGAC GTGGTAAAGG TGA CTCTGGA  
CAACTCCCAG GACACATAAA AAAGGTCCTG CACCATTTC ACTGAGACCT

2851 TGTTCAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC  
ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG

2901 TGCAGAGCTT CATGCTGCGG GGTGGTGTG TAGATGATCC AGTCGTAGCA  
ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT

2951 GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTCACTAGC AAGCTGATTG  
CCTCGCGACC CGCACCACGG ATTTTACAG AAAGTCATCG TTCGACTAAC

3001 CCAGGGGCAG GCCCTTGGTG TAAGTGTTA CAAAGCGGT AAGCTGGGAT  
GGTCCCCGTC CGGGAACCAC ATTCACAAAT GTTTCGCCAA TTCGACCCTA

3051 GGTGTCATAC GTGGGGATAT GAGATGCATC TTGGACTGTA TTTT TAGGT  
CCCACGTATG CACCCCTATA CTCTACGTAG AACCTGACAT AAAATCCAA

3101 GGCTATGTTC CCAGCCATAT CCCTCCGGGG ATTCATGTTG TGCAGAACCA  
CCGATACAAG GTTCGGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT

3151 CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTCATG TAGCTTAGAA  
GGTCGTGTCA CATAGGCCAC GTGAACCCTT TAAACAGTAC ATCGAATCTT

3201 GGAAATGCGT GGAAGAACTT GGAGACGCC TTGTGACCTC CAAGATTTTC  
CCTTTACGCA CCTTCTTGAA CCTCTGCGGG AACACTGGAG GTTCTAAAG

3251 CATGCATTTCG TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCCTGGG  
GTACGTAAGC AGGTATTACT ACCGTTACCC GGGTGCCCCG CGCCGGACCC

3301 CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTT CAGGATGAGA  
GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCCTACTCT

3351 TCGTCATAGG CCATTTTAC AAAGCGCGG CGGAGGGTGC CAGACTGCGG  
AGCAGTATCC GGTAAAAATG TTTCGCGCCC GCCTCCACG GTCTGACGCC

3401 TATAATGGTT CCATCCGGCC CAGGGGCGTA GTTACCCTCA CAGATTTGCA  
ATATTACCAA GGTAGGCCGG GTCCCCGCAT CAATGGGAGT GTCTAAACGT

3451 TTTCCACGC TTTGAGTTCA GATGGGGGA TCATGTCTAC CTGCGGGGCG  
AAAGGGTGCG AAAC TCAAGT CTACCCCTT AGTACAGATG GACGCCCCGC

3501 ATGAAGAAAA CGGTTTCCGG GGTAGGGGAG ATCAGCTGGG AAGAAAGCAG  
TACTTCTTTT GCCAAAGGCC CCATCCCTC TAGTCGACCC TTCTTTCGTG

3551 GTTCCTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCC TAAATCACAC  
CAAGGACTCG TCGACGCTGA ATGGCGTCGG CCACCCGGGC ATTTAGTGTG

3601 CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTCATCC  
GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTCGA CGGCAGTAGG

3651 CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTC  
GACTCGTCCC CCCGGTGAAG CAATTCGTAC AGGGACTGAG CGTACAAAAG

Figure 27D



3701 CCTGACCAAA TCCGCCAGAA GGCCTCGCC GCCCAGCGAT AGCAGTTCTT  
GGACTGGTTT AGGCGGTCTT CCGCAGCGG CGGGTCGCTA TCGTCAAGAA

3751 GCAAGGAAGC AAAGTTTTTC AACGGTTTGA GACCGTCCGC CGTAGGCATG  
CGTTCCTTCG TTCAAAAAG TTGCCAAACT CTGGCAGGCG GCATCCGTAC

3801 CTTTTGAGCG TTGACCAAG CAGTTCCAGG CGGTCCCACA GCTCGGTCAC  
GAAAACCTCG AAACTGGTTC GTCAAGGTCC GCCAGGGTGT CGAGCCAGTG

3851 CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG  
GACGAGATGC CGTAGAGCTA GGTCTATAG AGGAGCAAAG CGCCCAACCC

3901 GCGGCTTTCG CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CGGGCCAGGG  
CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGGTCCC

3951 TCATGTCTTT CCACGGGCGC AGGGTCCTCG TCAGCGTAGT CTGGGTCACG  
AGTACAGAAA GGTGCCCGCG TCCCAGGAGC AGTCGCATCA GACCCAGTGC

4001 GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GCTTGAGGCT  
CACTTCCCA CGCGAGGCC GACGCGCGAC CGGTCCCACG CGAACTCCGA

4051 GGTCTGCTG GTGCTGAAGC GCTGCCGGTC TTCGCCCTGC GCGTCGGCCA  
CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGGT

4101 GG TAGCATTT GACCATGGTG TCATAGTCCA GCCCCTCCGC GCGTGGCCC  
CCATCGTAAA CTGGTACCAC AGTATCAGGT CGGGGAGGCG CCGCACCGGG

4151 TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG  
AACCGCGCGT CGAACGGGAA CCTCTCCGC GCGTGCTCC CCGTCACGTC

4201 ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT  
TGAAAACCTCC CGCATCTCGA ACCCGCGCTC TTTATGGCTA AGGCCCTCA

4251 AGGCATCCGC GCCGCAGGCC CCGCAGACGG TCTCGCATTC CACGAGCCAG  
TCCGTAGGCG CGGCGTCCGG GCGTCTGCC AGAGCGTAAG GTGCTCGGTC

4301 GTGAGCTCTG GCCGTTCGGG GTCAAAAACC AGGTTTCCCC CATGCTTTTT  
CACTCGAGAC CGGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA

4351 GATGCGTTTC TTACCTCTGG TTTCCATGAG CCGGTGTCCA CGCTCGGTGA  
CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT

4401 CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCTCG  
GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAACCTCTC GGACAGGAGC

4451 AGCGGTGTTC CGCGGTCTCT CTCGTATAGA AACTCGGACC ACTCTGAGAC  
TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG

4501 AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC  
TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTACAC CTCCCCATCG

4551 GGTCTGTGTC CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG  
CCAGCAACAG GTGATCCCC AGGTGAGCGA GGTCCACAC TTCTGTGTAC

4601 TCGCCCTCTT CGGCATCAAG GAAGGTGATT GGTGTGTAGG TG TAGGCCAC  
AGCGGGAGAA GCCGTAGTTC CTTCCACTAA CCAAACATCC ACATCCGGTG

Figure 27E

4701 CGTCCTCACT CTCTTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT  
GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA

4751 GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCACT  
CTCATGAGGG AGACTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA

4801 TTCCAAAAAC GAGGAGGATT TGATATTAC CTGGCCCGCG GTGATGCCTT  
AAGGTTTTTG CTCCTCCTAA ACTATAAGTG GACCGGGCGC CACTACGGAA

4851 TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTTGTTGTCA  
ACTCCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT

4901 AGCTTGGTGG CAAACGACCC GTAGAGGGCG TTGGACAGCA ACTTGGCGAT  
TCGAACCACC GTTTGCTGGG CATCTCCCGC AACCTGTCGT TGAACCGCTA

4951 GGAGCGCAGG GTTTGGTTT TGTCGCGATC GCGCGCTCC TTGGCCGCGA  
CCTCGCGTCC CAAACCAAAA ACAGCGCTAG CCGCGCGAGG AACC GGCGCT

5001 TGTTTAGCTG CACGTATTCG CGCGCAACGC ACCGCCATTC GGGAAAGACG  
ACAAATCGAC GTGCATAAGC GCGCGTTGCG TGGCGGTAAG CCCTTTCTGC

5051 GTGGTGCCT CGTCGGGCAC CAGGTGCACG CGCCAACCGC GGTGTGCAG  
CACCACGCGA GCAGCCCGTG GTCCACGTGC GCGGTTGGCG CCAACACGTC

5101 GGTGACAAGG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG  
CCACTGTTCC AGTTGCGACC ACCGATGGAG AGGCGCATCC GCGAGCAACC

5151 TCCAGCAGAG GCGGCCGCCC TTGCGCGAGC AGAATGGCGG TAGGGGGTCT  
AGGTCGTCTC CGCCGGCGGG AACGCGCTCG TCTTACCGCC ATCCCCCAGA

5201 AGCTGCGTCT CGTCCGGGGG GTCTGCGTCC ACGGTAAAGA CCCC GGCGC  
TCGACGCAGA GCAGGCCCCC CAGACGCAGG TGCCATTTCT GGGGGCCGTC

5251 CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT  
GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTTT AGATCGCGGA

5301 GCTGCCATGC GCGGGCGGCA AGCGCGCGCT CGTATGGGTT GAGTGGGGGA  
CGACGGTACG CGCCCGCCGT TCGCGCGCGA GCATACCCAA CTCACCCCT

5351 CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC  
GGGGTACCGT ACCCCACCCA CTCGCGCCTC CGCATGTACG GCGTTTACAG

5401 GTAAACGTAG AGGGCTCTC TGAGTATTCC AAGATATGTA GGGTAGCATC  
CATTTGCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG

5451 TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA  
AAGGTGGCGC CTACGACCGC GCGTGCTTA GCATATCAAG CACGCTCCCT

5501 GCGAGGAGGT CGGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA  
CGCTCCTCCA GCCCTGGCTC CAACGATGCC CGCCCGACGA GACGAGCCTT

5551 GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGGACGCT  
CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTGCGA

Figure 27F

5651 GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC  
CTCCGCATCC TCAGCGCGTC GAACAACCTGG TCGAGCCGCC ACTGGACGTG

5701 GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT  
CAGATCCCGC GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA

5751 GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAACTC TTCGCGGTCT  
CAGGGAAAAA AAAGGTGTCT AGCGCCAACCT CCTGTTTGAG AAGCGCCAGA

5801 TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC  
AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCTCGG

5851 TAGCATGTAG AACTGGTTGA CGGCCTGGTA GGCGCAGCAT CCCTTTTCTA  
ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT

5901 CGGGTAGCGC GTATGCCTGC GCGGCCTTCC GGAGCGAGGT GTGGGTGAGC  
GCCCATCGCG CATACGGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG

5951 GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT  
CGTTTCCACA GGGACTGGTA CTGAACTCC ATGACCATAA ACTTCAGTCA

6001 GTCGTGCGAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTGG  
CAGCAGCGTA GCGGGGACGA GGGTCTCGTT TTTCAGGCAC GCGAAAAACC

6051 AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC  
TTGCGCCTAA ACCGTCCCGC TTCCACTGTA GCAACTTCTC ATAGAAAGGG

6101 GCGCGAGGCA TAAAGTTGCG TGTGATGCGG AAGGGTCCCG GCACCTCGGA  
CGCGCTCCGT ATTTCAACGC ACACTACGCC TTCCCAGGGC CGTGGAGCCT

6151 ACGGTTGTTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTTGA  
TGCCAACAAT TAATGGACCC GCCGCTCGTG CTAGAGCAGT TTCGGCAACT

6201 TGTTGTGGCC CACAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG  
ACAACACCGG GTGTTACATT TCAAGGTCTT TCGCGCCCTA CGGGAACTAC

6251 GAAAGGCAATT TTTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG  
CTTLCGTTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC

6301 CCCGTGCTCT GAAAGGGCCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA  
GGGCACGAGA CTTTCCCGGG TCAGACGTTT TACTCCCAAC CTTCGCTGCT

6351 ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAAG  
TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC

6401 GTCCTAAACT GGCGACCTAT GGCCATTTTT TCTGGGGTGA TGCAGTAGAA  
CAGGATTTGA CCGCTGGATA CCGGTAAAAA AGACCCCACT ACGTCATCTT

6451 GGTAAGCGGG TCTTGTTCCC AGCGGTCCCA TCCAAGGTTT CCGGCTAGGT  
CCATTGCCCC AGAACAAGGG TCGCCAGGGT AGGTTCCAAG CGCCGATCCA

6501 CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACCT CATGACCAGC  
GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTCG

Figure 27G

6601 TACATCGTAG GTGACAAAGA GACGCTCGGT GCGAGGATGC GAGCCGATCG  
ATGTAGCATC CACTGTTTCT CTGCGAGCCA CGCTCCTACG CTCGGCTAGC

6651 GGAAGAACTG GATCTCCCGC CACCAATTGG AGGAGTGGCT ATTGATGTGG  
CCTTCTTGAC CTAGAGGGCG GTGGTTAACC TCCTCACCGA TAACTACACC

6701 TGAAAGTAGA AGTCCCTGCG ACGGGCCGAA CACTCGTGCT GGCTTTTGTA  
ACTTTCATCT TCAGGGACGC TGCCCGGCTT GTGAGCACGA CCGAAAACAT

6751 AAAACGTGCG CAGTACTGGC AGCGGTGCAC GGGCTGTACA TCCTGCACGA  
TTTTGCACGC GTCATGACCG TCGCCACGTG CCCGACATGT AGGACGTGCT

6801 GGTTGACCTG ACGACCGCGC ACAAGGAAGC AGAGTGGGAA TTTGAGCCCC  
CCAACTGGAC TGCTGGCGCG GTTTCCTTCG TCTCACCTT AACTCGGGG

6851 TCGCCTGGCG GGTTTGGCTG GTGGTCTTCT ACTTCGGCTG CTTGTCTTGG  
AGCGGACCGC CCAAACCGAC CACCAGAAGA TGAAGCCGAC GAACAGGAAC

6901 ACCGTCTGGC TGCTCGAGGG GAGTTACGGT GGATCGGACC ACCACGCCGC  
TGGCAGACCG ACGAGCTCCC CTCAATGCCA CCTAGCCTCG TGGTGCGGCG

6951 GCGAGCCCAA AGTCCAGATG TCCGCGCGCG GCGGTGCGAG CTTGATGACA  
CGCTCGGGTT TCAGGTCTAC AGGCGCGCGC CGCCAGCCTC GAACTACTGT

7001 ACATCGCGCA GATGGGAGCT GTCCATGGTC TGGAGCTCCC GCGGCGTCAG  
TGTAGCGCGT CTACCCTCGA CAGGTACCAG ACCTCGAGGG CGCCGCAGTC

7051 GTCAGGCGGG AGCTCCTGCA GGTTTACCTC GCATAGACGG GTCAGGGCGC  
CAGTCCGCCC TCGAGGACGT CCAAATGGAG CGTATCTGCC CAGTCCCGCG

7101 GGGCTAGATC CAGGTGATAC CTAATTTCCTA GGGGCTGGTT GGTGGCGGCG  
CCCGATCTAG GTCCACTATG GATTAAAGGT CCCCACCAA CCACCGCCGC

7151 TCGATGGCTT GCAAGAGGCC GCATCCCCGC GGCGCGACTA CGGTACCGCG  
AGCTACCGAA CGTTCTCCGG CGTAGGGGCG CCGCGCTGAT GCCATGGCGC

7201 CGGCGGGCGG TGGGCCGCGG GGGTGTCTTT GGATGATGCA TCTAAAAGCG  
GCCGCCCGCC ACCCGGCGCC CCCACAGGAA CCTACTACGT AGATTTTCGC

7251 GTGACGCGGG CGAGCCCCCG GAGGTAGGGG GGGCTCCGGA CCCGCCGGGA  
CACTGCGCCC GCTCGGGGGC CTCCATCCCC CCCGAGGCCT GGGCGGCCCT

7301 GAGGGGGCAG GGGCACGTCG GCGCCGCGCG CCGGCAGGAG CTGGTGCTGC  
CTCCCCGTC CCCGTGCAGC CGCGGCGCGC GCGCGTCTC GACCACGACG

7351 GCGCGTAGGT TGCTGGCGAA CGCGACGACG CGGCGGTTGA TCTCCTGAAT  
CGCGCATCCA ACGACCGCTT GCCTGCTGC GCCGCCAAT AGAGGACTTA

7401 CTGGCGCCTC TGCGTGAAGA CGACGGGCCC GGTGAGCTTG AACCTGAAAG  
GACCGCGGAG ACGCACTTCT GTGCCCCGGG CCACTCGAAC TTGGACTTTC

7451 AGAGTTCGAC AGAATCAATT TCGGTGTCGT TGACGGCGGC CTGGCGCAA  
TCTCAAGCTG TCTTAGTTAA AGCCACAGCA ACTGCCGCG GACCGCGTTT

Figure 27H

7551 CTGCTCGATC TCTTCCTCCT GGAGATCTCC GCGTCCGGCT CGCTCCACGG  
GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCC

7601 TGGCGGCGAG GTCGTTGGAA ATGCGGGCCA TGAGCTGCGA GAAGGCGTTG  
ACCGCCGCTC CAGCAACCTT TACGCCCGGT ACTCGACGCT CTTCCGCAAC

7651 AGGCCTCCCT CGTTCCAGAC GCGGCTGTAG ACCACGCCCC CTTCCGGCATC  
TCCGGAGGGA GCAAGGTCTG CGCCGACATC TGGTGCGGGG GAAGCCGTAG

7701 GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCACG TGCCGGGCGA  
CGCCCGCGCG TACTGGTGGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT

7751 AGACGGCGTA GTTTCGCAGG CGCTGAAAGA GGTAGTTGAG GGTGGTGGCG  
TCTGCCGCAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC

7801 GTGTGTTCTG CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTC  
CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCAGCGT TGCACCTAAG

7851 GTTGATATCC CCCAAGGCCT CAAGGCGCTC CATGGCCTCG TAGAAGTCCA  
CAACTATAGG GGGTTCGGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT

7901 CGGCGAAGTT GAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC  
GCCGCTCAA CTTTTTGACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG

7951 TCCAGAAGAC GGATGAGCTC GCGGACAGTG TCGCGCACCT CGCGCTCAAA  
AGGTCTTCTG CCTACTCGAG CCGCTGTCAC AGCGCGTGGA GCGCGAGTTT

8001 GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCCTCTTCC ATAAGGGCCT  
CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTCCCGGA

8051 CCCCTTCTTC TTCTTCTGGC GCGGTTGGGG GAGGGGGGAC ACGGCGGCGA  
GGGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCCCCTG TGCCGCCGCT

8101 CGACGGCGCA CCGGGAGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG  
GCTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC

8151 GCGACGGCGC ATGGTCTCGG TGACGGCGCG GCCGTTCTCG CGGGGGCGCA  
CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCCCCCGCGT

8201 GTTGGAAGAC GCCGCCCGTC ATGTCCCGGT TATGGGTTGG CGGGGGGCTG  
CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCCAACC GCCCCCGAC

8251 CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTTGTGT  
GGTACGCCGT CCCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA

8301 AGGTACTCCG CCGCCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG  
TCCATGAGGC GCGGGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC

8351 AAAACCTCTC GAGAAAGGCG TCTAACCAGT CACAGTCGCA AGGTAGGCTG  
TTTTGGAGAG CTCTTTCCGC AGATTGGTCA GTGTCAGCGT TCCATCCGAC

8401 AGCACCCTGG CGGGCGGCAG CGGGCGGCGG TCGGGGTTGT TTCTGGCGGA  
TCGTGGCACC GCCCGCCGTC GCCCGCCGCC AGCCCCAACA AAGACCGCCT

Figure 27I

8501 TCGACAGAAG CACCATGTCC TTGGGTCCGG CCTGCTGAAT GCGCAGGCGG  
 AGCTGTCTTC GTGGTACAGG AACCCAGGCC GGACGACTTA CGCGTCCGGC  
 8551 TCGGCCATGC CCCAGGCTTC GTTTTGACAT CGGCGCAGGT CTTTGTAGTA  
 AGCCGGTACG GGGTCCGAAG CAAAACGTGA GCCGCGTCCA GAAACATCAT  
 8601 GTCTTGCAATG AGCCTTTCTA CCGGCACTTC TTCTTCTCCT TCCTCTTGTC  
 CAGAACGTAC TCGGAAAGAT GGCCGTGAAG AAGAAGAGGA AGGAGAACAG  
 8651 CTGCATCTCT TGCATCTATC GCTGCGGCGG CGGCGGAGTT TGGCCGTAGG  
 GACGTAGAGA ACGTAGATAG CGACGCCGCC GCCGCCTCAA ACCGGCATCC  
 8701 TGGCGCCCTC TTCCTCCCAT GCGTGTGACC CCGAAGCCCC TCATCGGCTG  
 ACCGCGGGAG AAGGAGGGTA CGCACACTGG GGCTTCGGGG AGTAGCCGAC  
 8751 AAGCAGGGCT AGGTCGGCGA CAACGCGCTC GGCTAATATG GCCTGCTGCA  
 TTCGTCCCGA TCCAGCCGCT GTTGC GCGAG CCGATTATAC CGGACGACGT  
 8801 CCTGCGTGAG GGTAGACTGG AAGTCATCCA TGTCCACAAA GCGGTGGTAT  
 GGACGCACTC CCATCTGACC TTCAGTAGGT ACAGGTGTTT CGCCACCATA  
 8851 GCGCCCGTGT TGATGGTGTA AGTGCACTTG GCCATAACGG ACCAGTTAAC  
 CGCGGGCACA ACTACCACAT TCACGTCAAC CGGTATTGCC TGGTCAATTG  
 8901 GSTCTGGTGA CCCGGCTGCG AGAGCTCGGT GTACCTGAGA CGCGAGTAAG  
 CCAGACCACT GGGCCGACGC TCTCGAGCCA CATGGACTCT GCGCTCATTG  
 8952 CCCTCGAGTC AAATACGTAG TCGTTGCAAG TCCGCACCAG GTACTGGTAT  
 GGGAGCTCAG TTTATGCATC AGCAACGTTT AGGCGTGGTC CATGACCATA  
 9001 CCCACCAAAA AGTGCGGCGG CGGCTGGCGG TAGAGGGGCC AGCGTAGGGT  
 GGGTGGTTTT TCACGCCGCC GCCGACCGCC ATCTCCCCGG TCGCATCCCA  
 9051 GGCCGGGGCT CCGGGGGCGA GATCTTCCAA CATAAGGCGA TGATATCCGT  
 CCGGCCCCGA GGCCCCGCT CTAGAAGGTT GTATTCCGCT ACTATAGGCA  
 9101 AGATGTACCT GGACATCCAG GTGATGCCGG CGGCGGTGGT GGAGGCGCGC  
 TCTACATGGA CCTGTAGGTC CACTACGGCC GCCGCCACCA CCTCCGCGCG  
 9151 GGAAAGTCGC GGACGCGGTT CCAGATGTTG CGCAGCGGCA AAAAGTGCTC  
 CCTTTTACGCG CCTGCGCCAA GGTCTACAAC GCGTCGCCGT TTTTCACGAG  
 9201 CATGGTCGGG ACGCTCTGGC CGGTCAGGCG CGCGCAATCG TTGACGCTCT  
 GTACCAGCCC TCGGAGACCG GCCAGTCCGC GCGCGTTAGC AACTGCGAGA  
 9251 AGACCGTGCA AAAGGAGAGC CTGTAAGCGG GCACTCTTCC GTGGTCTGGT  
 TCTGGCACGT TTTCTCTCG GACATTGCGC CGTGAGAAGG CACCAGACCA  
 9301 GGATAAATTC GCAAGGGTAT CATGGCGGAC GACCGGGGTT CGAGCCCCGT  
 CCTATTTAAG CGTTCCCATTA GTACCGCCTG CTGGCCCCAA GCTCGGGGCA  
 9351 ATCCGGCCGT CCGCCGTGAT CCATGCGGTT ACCGCCCGCG TGTCGAACCC  
 TAGGCCGGCA GCGGCGACTA GGTACGCCAA TGGCGGGCGC ACAGCTTGGG

Figure 27J

9451 GGC GCGCGCGG CTGCTGCGCT AGCTTTT TTG GCCACTGGCC GCGCGCAGCG  
CCGCGCCGCC GACGACGCGA TCGAAAAAAC CGGTGACCGG CCGCGCTCGC

9501 TAAGCGGTTA GGCTGGAAAG CGAAAGCATT AAGTGGCTCG CTCCCTGTAG  
ATTCGCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC

9551 CCGGAGGGTT ATTTTCCAAG GGTGAGTCG CGGGACCCCC GGTTCGAGTC  
GGCCTCCCAA TAAAAGGTTT CCAACTCAGC GCCCTGGGGG CCAAGCTCAG

9601 TCGGACCGGC CGGACTGCGG CGAACGGGGG TTTGCCTCCC CGTCATGCAA  
AGCCTGGCCG GCCTGACGCC GCTTGCCCCC AAACGGAGGG GCAGTACGTT

9651 GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTTGCT  
CTGGGGCGAA CGTTTAAGGA GGCCTTGTG CTTGCTCGGG GAAAAACGA

9701 TTTCCCAGAT GCATCCGGTG CTGCGGCAGA TCGCCCCCCC TCCTCAGCAG  
AAAGGGTCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGGG AGGAGTCGTC

9751 CGGCAAGAGC AAGAGCAGCG GCAGACATGC AGGGCACCCCT CCCCTCCTCC  
GCCGTTCTCG TTCTCGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG

9801 TACCGCGTCA GGAGGGGCGA CATCCGCGGT TGACGCGGCA GCAGATGGTG  
ATGGCGCAGT CCTCCCCGCT GTAGGCGCCA ACTGCGCCGT CGTCTACCAC

9851 ATTACGAACC CCCGCGGCGC CGGGCCCGGC ACTACCTGGA CTTGGAGGAG  
TAATGCTTGG GGGCGCCGCG GCCCGGGCCG TGATGGACCT GAACCTCCTC

9901 GCGGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCCTGAGC GGCACCCAAG  
CCGCTCCCGG ACCGCGCCGA TCCTCGCGGG AGAGGACTCG CCGTGGGTTC

9951 GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACC  
CCACGTCGAC TTCGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG

10001 TGTTCGCGA CCGCGAGGGA GAGGAGCCCG AGGAGATGCG GGATCGAAAG  
ACAAAGCGCT GCGCTCCCT CTCTCGGGC TCCTCTACGC CCTAGCTTTC

10051 TTCCACGCAG GCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGGTTGCT  
AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA

10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACC GGGATT AGTCCCGCGC  
CGCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG

10151 GCGCACACGT GCGGCGCGCC GACCTGGTAA CCGCATACGA GCAGACGGTG  
CGCGTGTGCA CCGCCGGCGG CTGGACCAAT GCGGTATGCT CGTCTGCCAC

10201 AACCAGGAGA TTAAC TTTCA AAAAAGCTTT AACCAACCAG TCGGTACGCT  
TTGGTCCTCT AATTGAAAGT TTTTTCGAAA TTGTTGGTGC ACGCATGCGA

10251 TGTGGCGCGC GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG  
ACACCGCGCG CTCCTCCACC GATATCCTGA CTACGTAGAC ACCCTGAAAC

10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GGCGCAGCTG  
ATTCGCGCGA CCTCGTTTTG GGT TATCGT TCGGCGAGTA CCGCGTCGAC

Figure 27K

10401 GCTAAACATA GTAGAGCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA  
CGATTTGTAT CATCTCGGGC TCCCGCGAC CGACGAGCTA AACTATTTGT

10451 TCCTGCAGAG CATAGTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG  
AGGACGTCTC GTATCACCAC GTCCTCGCGT CGAACTCGGA CCGACTGTTC

10501 GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCC  
CACCGGCGGT AGTTGATAAG GTACGAATCG GACCCGTTCA AAATGCGGGC

10551 CAAGATATAC CATACCCTT ACCTTCCCAT AGACAAGGAG GTAAAGATCG  
GTTCTATATG GTATGGGAA TGCAAGGGTA TCTGTTCTTC CATTTCTAGC

10601 AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC  
TCCCAAGAT GTACGCGTAC CCGACTTCC ACGAATGGAA CTCGCTGCTG

10651 CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG  
GACCCGCAA TAGCGTTGCT CGCGTAGGTG TTCCGGCACT CGCACTCGGC

10701 GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC  
CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTCGGAC GTTTCCCGGG

10751 TGGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCCTA CTTTGACGCG  
ACCGACCGTG CCCGTCGCCG CTATCTCTCC GGCTCAGGAT GAAACTGCGC

10801 GCGGCTGACC TGGCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG  
CCGCGACTGG ACGCGACCCG GGGTTCGGCT GCGCGGGACC TCCGTCGACC

10851 GGCCGGACCT GGGCTGGCGG TGGCACC CGC GCGCTGGC AACGTCGGCG  
CCGGCCTGGA CCCGACCGCC ACCGTGGGCG CGCGCGACC TTGCAGCCGC

10901 GCGTGAGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG  
CGCACCTCCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CCTGCCGCTC

10951 TACTAAGCGG TGATGTTTCT GATCAGATGA TGCAAGACGC AACGGACCCG  
ATGATTCGCC ACTACAAAGA CTAGTCTACT ACGTTCTGCG TGCCCTGGGC

11001 GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA  
CGCCACGCCC GCGCGACGT CTCGGTCGGC AGGCCGGAAT TGAGGTGCCT

11051 CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC  
GCTGACCGCG GTCCAGTACC TGGCGTAGTA CAGCGACTGA CGCGCGTTAG

11101 CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG  
GACTGCGCAA GGCCGTCGTC GCGCTCCGGT TGGCCGAGAG GCGTTAAGAC

11151 GAAGCGGTGG TCCCGGCGCG CGCAAACCCC ACGCACGAGA AGGTGCTGGC  
CTTCGCCACC AGGGCCGCGC GCGTTTGGGG TGCGTGCTCT TCCACGACCG

11201 GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG  
CTAGCATTG CGCGACCGGC TTTTGTCCCG GTAGGCCGGG CTGCTCCGGC

11251 GCCTGGTCTA CGACGCGCTG CTTAGCGCG TGGCTCGTTA CAACAGCGGC  
CGGACCAGAT GCTGCGCGAC GAAGTCGCGC ACCGAGCAAT GTTGTCGCCG

Figure 27L



11351 GGCGCAGCGT GAGCGCGCGC AGCAGCAGGG CAACCTGGGC TCCATGGTTG  
CCGCGTCGCA CTCGCGCGCG TCGTCGTCCC GTTGGACCCG AGGTACCAAC

11401 CACTAAACGC CTTCTGAGT ACACAGCCCG CCAACGTGCC GCGGGGACAG  
GTGATTTGCG GAAGGACTCA TGTGTCGGGC GGTTCACGG CGCCCTGTCT

11451 GAGGACTACA CCAACTTTGT GAGCGCACTG CGGCTAATGG TGACTGAGAC  
CTCCTGATGT GGTGAAACA CTCGCGTGAC GCCGATTACC ACTGACTCTG

11501 ACCGCAAAGT GAGGTGTACC AGTCTGGGCC AGACTATTTT TTCCAGACCA  
TGGCGTTTCA CTCCACATGG TCAGACCCGG TCTGATAAAA AAGGTCTGGT

11551 GTAGACAAGG CCTGCAGACC GTAAACCTGA GCCAGGCTTT CAAAACTTG  
CATCTGTTCC GGACGTCTGG CATTTGGACT CGGTCCGAAA GTTTTTGAAC

11601 CAGGGGCTGT GGGGGGTGCG GGCTCCACACA GGCGACCGCG CGACCGTGTC  
GTCCCCGACA CCCCCACGC CCGAGGGTGT CCGCTGGCGC GCTGGCACAG

11651 TAGCTTGCTG ACGCCCAACT CGCGCCTGTT GCTGCTGCTA ATAGCGCCCT  
ATCGAACGAC TCGGGGTGA GCGCGGACAA CGACGACGAT TATCGCGGGA

11701 TCACGGACAG TGGCAGCGTG TCCCGGGACA CATACTAGG TCACTTGCTG  
AGTGCCTGTC ACCGTCGCAC AGGGCCCTGT GTATGGATCC AGTGAACGAC

11751 ACACTGTACC GCGAGGCCAT AGGTCAGGCG CATGTGGACG AGCATACTTT  
TGTGACATGG CGCTCCGGTA TCCAGTCCGC GTACACCTGC TCGTATGAAA

11801 CCAGGAGATT ACAAGTGTC ACGCGCGCCT GGGGCAGGAG GACACGGGCA  
GGTCTCTTAA TGTTACAGT CGGCGCGCGA CCCCCTCTC CTGTGCCCGT

11851 GCCTGGAGGC AACCCTAAAC TACCTGCTGA CCAACCGGCG GCAGAAGATC  
CGGACCTCCG TTGGGATTG ATGGACGACT GGTGCGCCG CGTCTTCTAG

11901 CCCTCGTTGC ACAGTTTAAA CAGCGAGGAG GAGCGCATTT TGCGCTACGT  
GGGAGCAACG TGTCAAATT GTGCTCTCTC CTCGCGTAAA ACGCGATGCA

11951 GCAGCAGAGC GTGAGCCTTA ACCTGATGCG CGACGGGGTA ACGCCAGCG  
CGTCTGCTCG CACTCGGAAT TGGACTACGC GCTGCCCCAT TGCGGGTCCG

12001 TGGCGCTGGA CATGACCGCG CGCAACATGG AACCGGGCAT GTATGCCTCA  
ACCGCGACCT GACTGGCGC GCGTTGTACC TTGGCCCGTA CACACGGAGT

12051 AACC GGCCGT TTATCAACCG CCTAATGGAC TACTTGATC GCGCGGCCG  
TTGGCCGGCA AATAGTTGGC GGATTACCTG ATGAACGTAG CGCGCCGGC

12101 CGTGAACCCC GAGTATTTCA CCAATGCCAT CTTGAACCCG CACTGGCTAC  
GCAC TTGGGG CTCATAAAGT GGTTACGGTA GAACTTGGGC GTGACCGATG

12151 CGCCCCCTGG TTTCTACACC GGGGGATTG AGGTGCCCCG GGGTAACGAT  
GCGGGGGACC AAAGATGTGG CCCCCTAAGC TCCACGGGCT CCCATTGCTA

12201 GGATTCTCTT GGGACGACAT AGACGACAGC GTGTTTTCCC CGCAACCGCA  
CCTAAGGAGA CCCTGCTGTA TCTGCTGTCG CACAAAAGGG GCGTTGGCGT

Figure 27 M

12301 AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC  
TCCTTTTCGAA GCGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG

12351 CCGCGGTCAG ATGCTAGTAG CCCATTTCCTA AGCTTGATAG GGTCTCTTAC  
GGCGCCAGTC TACGATCATC GGGTAAAGGT TCGAACTATC CCAGAGAATG

12401 CAGCACTCGC ACCACCCGCC CGCGCCTGCT GGGCGAGGAG GAGTACCTAA  
GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCCTC CTCATGGATT

12451 ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATT  
TGTTGAGCGA CGACGTCGGC GTCGCGCTTT TTTTGGACGG AGGCCGTAAA

12501 CCCAACAACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC  
GGGTTGTTGC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTTCTG

12551 GTACGCGCAG GAGCACAGGG ACGTGCCAGG CCCGCGCCCG CCCACCCGTC  
CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGCAG

12601 GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG  
CAGTTTCCGT GCTGGCAGTC GCCCCAGACC ACACCTCCT GCTACTGAGC

12651 GCAGACGACA GCAGCGTCCT GGATTGGA GGGAGTGGCA ACCCGTTTGC  
CGTCTGCTGT CGTCGCAGGA CCTAAACCTT CCTCACCGT TGGGCAAACG

12701 GCACCTTCGC CCCAGGCTGG GGAGAATGTT TTAACAAAAA AAAAAGCATG  
CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTTCTT TTTTTCGTAC

12751 ATGCAAAATA AAAAATCAC CAAGGCCATG GCACCGAGCG TTGGTTTCT  
TACGTTTTAT TTTTGTAGTG GTTCCGGTAC CGTGGCTCGC AACCAGAAAG

12801 TGTATTCCCC TAGTATGCG GCGCGCGGCG ATGTATGAGG AAGGTCCTCC  
ACATAAGGGG AATCATAAGC CGCGCGCCGC TACATACTCC TTCCAGGAGG

12851 TCCCTCCTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGGCGCTGG  
AGGGAGGATG CTCTACACC ACTCGCGCCG CGGTACCGC CGCCGCGACC

12901 GTTCTCCCTT CGATGCTCCC CTGGACCGC CGTTTGTGCC TCCGCGGTAC  
CAAGAGGGAA GCTACGAGG GACCTGGGCG GCAAACACGG AGGCGCCATG

12951 CTGCGGCCCTA CCGGGGGGAG AAACAGCATC CGTTACTCTG AGTTGGCACC  
GACGCCGGAT GGCCCCCTC TTTGTCTAGT GCAATGAGAC TCAACCGTGG

13001 CCTATTCGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG  
GGATAAGCTG TGGTGGGCAC ACATGGACCA CCTGTTGTTT AGTTGCCTAC

13051 TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC  
ACCGTAGGGA CTTGATGGTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG

13101 ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA  
TAAGTTTGT TACTGATGTC GGGCCCCCTC CGTTCGTGTG TCTGGTAGTT

13151 TCTTGACGAC CGGTCGCACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA  
AGAAGTCTG GCCAGCGTGA CCCCCTGCT GGAATTTTGG TAGGACGTAT

Figure 27N

13251 CGGGTGATGG TGTCGCGCTT GCCTACTAAG GACAATCAGG TGGAGCTGAA  
GCCCACTACC ACAGCGCGAA CGGATGATTC CTGTTAGTCC ACCTCGACTT

13301 ATACGAGTGG GTGGAGTTCA CGCTGCCCCG GGGCAACTAC TCCGAGACCA  
TATGCTCACC CACCTCAAGT GCGACGGGCT CCCGTTGATG AGGCTCTGGT

13351 TGACCATAGA CCTTATGAAC AACGCGATCG TGGAGCACTA CTTGAAAGTG  
ACTGGTATCT GGAATACTTG TTGCGCTAGC ACCTCGTGAT GAACTTTCAC

13401 GGCAGACAGA ACGGGGTTCT GGAAAGCGAC ATCGGGGTAA AGTTTGACAC  
CCGTCTGTCT TGCCCCAAGA CCTTTCGCTG TAGCCCCATT TCAAACGTG

13451 CCGCAACTTC AGACTGGGGT TTGACCCCGT CACTGGTCTT GTCATGCCTG  
GGCGTTGAAG TCTGACCCCA AACTGGGGCA GTGACCAGAA CAGTACGGAC

13501 GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT GCTGCCAGGA  
CCCATATATG TTTGCTTCGG AAGGTAGGTC TGTAATAAAA CGACGGTCCT

13551 TGCGGGGTGG ACTTCACCCA CAGCCGCTG AGCAACTTGT TGGGCATCCG  
ACGCCCCACC TGAAGTGGGT GTCGCGGGAC TCGTTGAACA ACCCGTAGGC

13601 CAAGCGGCAA CCTTCCAGG AGGGCTTTAG GATCACCTAC GATGATCTGG  
GTTCCCGTT GGGAAAGGTCC TCCCGAAATC CTAGTGGATG CTACTAGACC

13651 AGGGTGGTAA CATTCCCGCA CTGTTGGATG TGGACGCTA CCAGGCGAGC  
TCCCACCATT GTAAGGGCGT GACAACCTAC ACCTGCGGAT GGTCGCTCG

13701 TTGAAAGATG ACACCGAACA GGGCGGGGGT GGCGCAGGCG GCAGCAACAG  
AACTTTCTAC TGTGGCTTGT CCCGCCCCCA CCGCGTCCGC CGTCGTTGTC

13751 CAGTGGCAGC GGCGCGGAAG AGAACTCCAA CGCGGCAGCC GCGGCAATGC  
GTCAACGTCG CCGCGCCTTC TCTTGAGGTT GCGCCGTCGG CGCCGTTACG

13801 AGCCGGTGGA GGACATGAAC GATCATGCCA TTCGCGGCGA CACCTTTGCC  
TCGGCCACCT CCTGTACTTG CTAGTACGGT AAGCGCCGCT GTGGAAACGG

13851 ACACGGGCTG AGGAGAAGCG CGCTGAGGCC GAAGCAGCGG CCGAAGCTGC  
TGTGCCCCGAC TCCTCTTCGC GCGACTCCGG CTTCGTCGCC GGCTTCGACG

13901 CGCCCCCGCT GCGCAACCCG AGGTCGAGAA GCCTCAGAAG AAACCGGTGA  
GCGGGGGCGA CGCGTTGGGC TCCAGCTCTT CGGAGTCTTC TTTGGCCACT

13951 TCAAACCCCT GACAGAGGAC AGCAAGAAAC GCAGTTACAA CCTAATAAGC  
AGTTTGGGGA CTGTCTCCTG TCGTTCTTTG CGTCAATGTT GGATTATTG

14001 AATGACAGCA CCTTCACCCA GTACCGCAGC TGGTACCTTG CATACAACTA  
TTACTGTCTG GGAAGTGGGT CATGGCGTCG ACCATGGAAC GTATGTTGAT

14051 CGGCGACCCCT CAGACCGGAA TCCGCTCATG GACCCTGCTT TGCATCCTG  
GCCGCTGGGA GTCTGGCCTT AGGCGAGTAC CTGGGACGAA ACGTGAGGAC

14101 ACGTAACCTG CGGCTCGGAG CAGGTCTACT GGTGCTTGCC AGACATGATG  
TGCATTGGAC GCCGAGCCTC GTCCAGATGA CCAGCAACGG TCTGTACTAC

Figure 270

14201 GGTGGGCGCC GAGCTGTTGC CCGTGCACTC CAAGAGCTTC TACAACGACC  
CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG

14251 AGGCCGTCTA CTCCCAACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG  
TCCGGCAGAT GAGGGTTGAG TAGGCGGTCA AATGGAGAGA CTGGGTGCAC

14301 TTCAATCGCT TTCCCGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCAC  
AAGTTAGCGA AAGGGCTCTT GGTCTAAAAC CGCGCGGGCG GTCGGGGGTG

14351 CATCACCACC GTCAGTGAAG ACCTTCCTGC TCTCACAGAT CACGGGACGC  
GTAGTGGTGG CAGTCACTTT TGCAAGGACG AGAGTGTCTA GTGCCCTGCG

14401 TACCGCTGCG CAACAGCATC GGAGGAGTCC AGCGAGTGAC CATTACTGAC  
ATGGCGACGC GTTGTCTGAG CCTCCTCAGG TCGCTCACTG GTAATGACTG

14451 GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC  
CGGTCTGCGG CGTGGACGGG GATGCAAATG TTCCGGGACC CGTATCAGAG

14501 GCCGCGCGTC CTATCGAGCC GCACTTTTTG AGCAAGCATG TCCATCCTTA  
CGGCGCGCAG GATAGCTCGG CGTGAAAAC TCGTTCGTAC AGGTAGGAAT

14551 TATCGCCCGC CAATAACACA GGCTGGGGCC TGGCTTCCC AAGCAAGATG  
ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC

14601 TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGCGCGG  
AAACCGCCCC GGTCTTTCG GAGGCTGGTT GTGGGTCACG CGCACGCGCC

14651 GCACTACCGC GCGCCCTGGG GCGCGCACAA ACGCGGCCGC ACTGGGCGCA  
CGTGATGGCG CGCGGGACCC CGCGCGTGT TCGCGCGGCG TGACCCGCGT

14701 CCACCGTCGA TGACGCCATC GACGCGGTGG TGGAGGAGGC GCGCAACTAC  
GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG

14751 ACGCCACGCG CGCCACCAGT GTCCACAGTG GACGCGGCCA TTCAGACCGT  
TCCGGGTGCG GCGGTGGTCA CAGGTGTCAC CTGCGCCGGT AAGTCTGGCA

14801 GGTGCGCGGA GCGCGCGCT ATGCTAAAAT GAAGAGACGG CGGAGGCGCG  
CCACGCGCCT CGGCGCGCA TACGATTTTA CTTCTCTGCC GCCTCCGCGC

14851 TAGCACGTCG CCACCGCCGC CGACCCGGCA CTGCGGCCCA ACGCGCGGCG  
ATCGTGACG GGTGGCGGCG GCTGGGCCGT GACGGCGGGT TGCGCGCCGC

14901 GCGGCCCTGC TTAACCGCGC ACCTCGCACC GGCCGACGGG CGGCCATGCG  
CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCCGGTACGC

14951 GGCCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA  
CCGGCGAGCT TCCGACCGGC GCCCATAACA GTGACACGGG GGGTCCAGGT

15001 GGCGACGAGC GGCCGCCGCA GCAGCCGCGG CCATTAGTGC TATGACTCAG  
CCGCTGCTCG CCGCGGCGGT CGTCGGCGCC GGTAATCACG ATACTGAGTC

15051 GGTGCGAGGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCCTGCG  
CCAGCGTCCC CGTTGCACAT AACCCACGCG CTGAGCCAAT CGCCGGACGC

Figure 27P

15151 ACTTAGACTC GTACTGTTGT ATGTATCCAG CGGCGGCGGC GCGCAACGAA  
TGAATCTGAG CATGACAACA TACATAGGTC GCCGCCGCCG CGCGTTGCTT

15201 GCTATGTCCA AGCGCAAAAT CAAAGAAGAG ATGCTCCAGG TCATCGCGCC  
CGATACAGGT TCGCGTTTTA GTTCTTCTC TACGAGGTCC AGTAGCGCGG

15251 GGAGATCTAT GGCCCCCGA AGAAGGAAGA GCAGGATTAC AAGCCCCGAA  
CCTCTAGATA CCGGGGGGCT TCTTCCTTCT CGTCCTAATG TTCGGGGCTT

15301 AGCTAAAGCG GGTCAAAAAG AAAAAGAAAG ATGATGATGA TGAAGTTGAC  
TCGATTTTCG CCAGTTTTTC TTTTCTTTC TACTACTACT ACTTGAAGTG

15351 GACGAGGTGG AACTGCTGCA CGCTACCGCG CCCAGGCGAC GGGTACAGTG  
CTGCTCCACC TTGACGACGT GCGATGGCGC GGGTCCGCTG CCCATGTCAC

15401 GAAAGGTCGA CGCGTAAAAC GTGTTTTGCG ACCCGGCACC ACCGTAGTCT  
CTTTCAGCT GCGCATTTTG CACAAAACGC TGGGCCGTGG TGGCATCAGA

15451 TTACGCCCGG TGAGCGCTCC ACCCGCACCT ACAAGCGCGT GTATGATGAG  
AATGCGGGCC ACTCGCGAGG TGGGCGTGGA TGTTGCGCA CATACTACTC

15501 GTGTACGGCG ACGAGGACCT GCTTGAGCAG GCCAACGAGC GCCTCGGGGA  
CACATGCCGC TGCTCCTGGA CGAACTCGTC CGGTTGCTCG CGGAGCCCCCT

15551 GTTTGCCTAC GGAAAGCGGC ATAAGGACAT GCTGGCGTTG CCCTGGACG  
CAAACGGATG CCTTTCGCCG TATTCCTGTA CGACCGCAAC GCGACCTGC

15601 AGGGCAACCC AACACCTAGC CTAAAGCCCG TAACACTGCA GCAGGTGCTG  
TCCCGTTGGG TTGTGGATCG GATTTCGGG ATTGTGACGT CGTCCACGAC

15651 CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCTAAAGC GCGAGTCTGG  
GGGCGCAAC GTGGCAGGCT TCTTTTCGCG CCGGATTTCTG CGCTCAGACC

15701 TGAAGTGGCA CCCACCGTGC AGCTGATGGT ACCCAAGCGC CAGCGACTGG  
ACTGAACCGT GGGTGGCACG TCGACTACCA TGGGTTCTCG GTGCTGAC

15751 AAGATGTCTT GGAAAAATG ACCGTGGAAC CTGGGCTGGA GCGGAGGTC  
TTCTACAGAA CCTTTTTTAC TGGCACCTTG GACCCGACCT CGGGCTCCAG

15801 CGCGTGGCG CAATCAAGCA GGTGGCGCCG GGAAGGCGG TGCAGACCGT  
GCGCACGCCG GTTAGTTCTG CCACCGCGG CCTGACCCGC ACCTCTGGCA

15851 GGACGTTTCTG ATACCCACTA CCAGTAGCAC CAGTATTGCC ACCGCCACAG  
CCTGCAAGTC TATGGGTGAT GGTATCTGTC GTCATAACGG TGGCGGTGTC

15901 AGGGCATGGA GACACAAACG TCCCCGGTTG CCTCAGCGGT GCGGATGCC  
TCCCGTACCT CTGTGTTTGC AGGGGCCAAC GGAGTCGCCA CCGCCTACGG

15951 GCGGTGACAG CGGTGCTGCG GCGCGCTCC AAGACCTCTA CGGAGGTGCA  
CGCCACGTCC GCCAGCGACG CCGGCGCAGG TTCTGGAGAT GCCTCCACGT

16001 AACGGACCCG TGGATGTTTC GCGTTTCAGC CCCCCGCGC CCGCGCCGTT  
TTGCCTGGGC ACCTACAAAG CGCAAAGTCG GGGGCGCGG GCGCGGCAA

Figure 27Q

16051 CGAGGAAGTA CGGCGCCGCC AGCGCGCTAC TGCCCGAATA TGCCCTACAT  
GCTCCTTCAT GCCGCGGCGG TCGCGCGATG ACGGGCTTAT ACGGGATGTA

16101 CCTTCCATTG CGCCTACCCC CGGCTATCGT GGCTACACCT ACCGCCCCAG  
GGAAGGTAAC GCGGATGGGG GCCGATAGCA CCGATGTGGA TGGCGGGGTC

16151 AAGACGAGCA ACTACCCGAC GCCGAACCAC CACTGGAACC CGCCGCCGCC  
TTCTGCTCGT TGATGGGCTG CGGCTTGGTG GTGACCTTGG GCGGCGGCGG

16201 GTCGCCGTCG CCAGCCCGTG CTGGCCCCGA TTTCCGTGCG CAGGGTGGCT  
CAGCGGCAGC GGTGCGGCAC GACCGGGGCT AAAGGCACGC GTCCACCGA

16251 CGCGAAGGAG GCAGGACCCT GGTGCTGCCA ACAGCGCGCT ACCACCCAG  
GCGCTTCCTC CGTCTGGGA CCACGACGGT TGTCGCGCGA TGGTGGGGTC

16301 CATCGTTTAA AAGCCGGTCT TTGTGGTTCT TGCAGATATG GCCCTCACCT  
GTAGCAAATT TTCGGCCAGA AACACCAAGA ACGTCTATAC CGGGAGTGGA

16351 GCCGCCTCCG TTTCCCGGTG CCGGGATTCC GAGGAAGAAT GCACCGTAGG  
CGGCGGAGGC AAAGGGCCAC GGCCCTAAGG CTCCTTCTTA CGTGGCATCC

16401 AGGGGCATGG CCGGCCACGG CCTGACGGGC GGCATGCGTC GTGCGACCA  
TCCCCGTACC GGCCGGTGCC GGACTGCCCC CCGTACGCAG CACGCGTGGT

16451 CCGGCGGCGG CGCGCGTCGC ACCGTCGCAT GCGCGGCGGT ATCCTGCCCC  
GGCCCGCGCC GCGCGCAGCG TGCGAGCGTA CCGCGCGCCA TAGGACGGGG

16501 TCCTTATTCC ACTGATCGCC GCGGCGATTG GCGCCGTGCC CGGAATTGCA  
AGGAATAAGG TGA CTAGCGG CGCCGCTAAC CCGGCACGG GCCTTAACGT

16551 TCCGTGGCCT TGCAGGCGCA GAGACACTGA TTA AAAACAA GTTGCATGTG  
AGGCACCGGA ACGTCCGCGT CTCTGTGACT AATTTTGT CAACGTACAC

16601 GAAAAATCAA AATAAAAAGT CTGGACTCTC ACGCTCGCTT GGTCTGTAA  
CTTTTAGTT TTATTTTCA GACCTGAGAG TGCGAGCGAA CCAGGACATT

16651 CTATTTTGTA GAATGGAAGA CATCAACTTT GCGTCTCTGG CCCC GCGACA  
GATAAACAT CTTACCTTCT GTAGTTGAAA CGCAGAGACC GGGGCGCTGT

16701 CGGCTCGCGC CCGTTCATGG GAAACTGGCA AGATATCGGC ACCAGCAATA  
GCCGAGCGCG GGCAAGTACC CTTTGACCGT TCTATAGCCG TGGTCGTTAT

16751 TGAGCGGTGG CGCCTTCAGC TGGGGCTCGC TGTGGAGCGG CATTAAAAAT  
ACTCGCCACC GCGGAAGTCG ACCCCGAGCG ACACCTCGCC GTAATTTTAA

16801 TTCGGTTCCA CCGTTAAGAA CTATGGCAGC AAGGCCTGGA ACAGCAGCAC  
AAGCCAAGGT GGCAATTCCT GATACCGTCG TTCCGGACCT TGTCGTCGTG

16851 AGGCCAGATG CTGAGGGATA AGTTGAAAGA GCAAAATTTT CAACAAAAGG  
TCCGGTCTAC GACTCCCTAT TCAACTTTCT CGTTTTAAAG GTTGTTTTCC

16901 TGGTAGATGG CCTGGCCTCT GGCATTAGCG GGGTGGTGA CCTGGCCAAC  
ACCATCTACC GGACCGGAGA CCGTAATCGC CCCACCACCT GGACCGGTTG

16951 CAGGCAGTGC AAAATAAGAT TAACAGTAAG CTTGATCCCC GCCCTCCCGT  
GTCCGTCACG TTTTATTCTA ATTGTCATTC GAACTAGGGG CGGGAGGGCA

Figure 27R

17051 AAAAGCGTCC GCGCCCCGAC AGGGAAGAAA CTCTGGTGAC GCAAATAGAC  
TTTTCGCAGG CGCGGGGCTG TCCCTTCTTT GAGACCACTG CGTTTATCTG

17101 GAGCCTCCCT CGTACGAGGA GGCCTAAAG CAAGGCCTGC CCACCACCCG  
CTCGGAGGGA GCATGCTCCT CCGTGATTTC GTTCCGGACG GGTGGTGGGC

17151 TCCCATCGCG CCCATGGCTA CCGGAGTGCT GGGCCAGCAC ACACCCGTAA  
AGGGTAGCGC GGGTACCGAT GGCCTCACGA CCCGGTCGTG TGTGGGCATT

17201 CGCTGGACCT GCCTCCCCC GCGACACCC AGCAGAAACC TGTGCTGCCA  
GCGACCTGGA CGGAGGGGGG CGGCTGTGGG TCGTCTTTGG ACACGACGGT

17251 GGCCCGACCG CCGTTGTTGT AACCCGTCCT AGCCGCGCGT CCCTGCGCCG  
CCGGGCTGGC GGCAACAACA TTGGGCAGGA TCGGCGCGCA GGGACGCGGC

17301 CGCCGCCAGC GGTCCGCGAT CGTTGCGGCC CGTAGCCAGT GGCAACTGGC  
GCGGCGGTG CAGGCGCTA GCAACGCCGG GCATCGGTCA CCGTTGACCG

17351 AAAGCACACT GAACAGCATC GTGGGTCTGG GGGTGCAATC CCTGAAGCGC  
TTTCGTGTGA CTTGTCTGTAG CACCCAGACC CCCACGTTAG GGACTTCGCG

17401 CGACGATGCT TCTGATAGCT AACGTGTCGT ATGTGTGTCA TGTATGCGTC  
GCTGCTACGA AGACTATCGA TTGCACAGCA TACACACAGT ACATACGCAG

17451 CATGTCGCCG CCAGAGGAGC TGCTGAGCCG CCGCGCGCCC GCTTTCCAAG  
GTACAGCGGC GGTCTCCTCG ACGACTCGGC GCGCGCGGG CGAAAGGTTT

17501 ATGGCTACCC CTTCGATGAT GCCGCACTGG TCTTACATGC ACATCTCGGG  
TACCGATGGG GAAGCTACTA CGGCGTCACC AGAATGTACG TGTAGAGCCC

17551 CCAGGACGCC TCGGAGTACC TGAGCCCCGG GCTGGTGCAG TTGCCCCGCG  
GGTCCTGCGG AGCCTCATGG ACTCGGGGCC CGACCACGTC AAACGGGCGC

17601 CCACCGAGAC GTACTTCAGC CTGAATAACA AGTTTAGAAA CCCCACGGTG  
GGTGGCTCTG CATGAAGTCG GACTTATTGT TCAAATCTTT GGGGTGCCAC

17651 GCGCCTACGC ACGACGTGAC CACAGACCGG TCCCAGCGTT TGACGCTGCG  
CGCGGATGCG TGCTGCACTG GTGTCTGGCC AGGGTCGCAA ACTGCGACGC

17701 GTTCATCCCT GTGGACCGTG AGGATACTGC GTACTCGTAC AAGGCGCGGT  
CAAGTAGGGA CACCTGGCAC TCCTATGACG CATGAGCATG TTCCGCGCCA

17751 TCACCCTAGC TGTGGGTGAT AACCGTGTGC TGGACATGGC TTCCACGTAC  
AGTGGGATCG ACACCCACTA TTGGCACACG ACCTGTACCG AAGGTGCATG

17801 TTTGACATCC GCGGCGTGCT GGACAGGGGC CCTACTTTTA AGCCCTACTC  
AAACTGTAGG CGCCGCACGA CCTGTCCCCG GGATGAAAAT TCGGGATGAG

17851 TGGCACTGCC TACAACGCCC TGGCTCCCAA GGGTGCCCCA AATCCTTGCG  
ACCGTGACGG ATGTTGCGGG ACCGAGGGTT CCCACGGGGT TTAGGAACGC

17901 AATGGGATGA AGCTGCTACT GCTCTTGAAT TAAACCTAGA AGAAGAGGAC  
TTACCCTACT TCGACGATGA CGAGAACTTT ATTTGGATCT TCTTCTCCTG

Figure 275

17951 GATGACAACG AAGACGAAGT AGACGAGCAA GCTGAGCAGC AAAAAACTCA  
CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTTTGAGT

18001 CGTATTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA  
GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCCTCCCAT

18051 TTCAAATAGG TGTGGAAGGT CAAACACCTA AATATGCCGA TAAACATTT  
AAGTTTATCC ACAGCTTCCA GTTTGTGGAT TTATACGGCT ATTTTGTAAG

18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA  
GTTGGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT

18151 TCATGCAGCT GGGAGAGTCC TAAAAAGAC TACCCCAATG AAACCATGTT  
AGTACGTCGA CCCTCTCAGG ATTTTTTCTG ATGGGGTTAC TTTGGTACAA

18201 ACGGTTTATA TGCAAAACCC ACAAATGAAA ATGGAGGGCA AGGCATTCTT  
TGCCAAGTAT ACGTTTTGGG TGTTTACTTT TACCTCCCGT TCCGTAAGAA

18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGGAAA TGCAATTTTT  
CATTTCTGTTG TTTTACCTTT CGATCTTTCA GTTCACCTTT ACGTTAAAAA

18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG  
GAGTTGATGA CTCCGTCGGC GTCCGTTACC ACTATTGAAC TGAGGATTTC

18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCAGA CACTCATATT  
ACCATAACAT GTCACCTCTA CATCTATATC TTTGGGGTCT GTGAGTATAA

18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA  
AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT

18451 ACAATCTATG CCCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTTA  
TGTTAGATAC GGGTTGTCCG GATTAATGTA ACGAAAATCC CTGTTAAAT

18501 TTGGTCTAAT GTATTACAAC AGCACGGGTA ATATGGGTGT TCTGGCGGGC  
AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG

18551 CAAGCATCGC AGTTGAATGC TGTGTAGAT TTGCAAGACA GAAACACAGA  
GTTCTGAGCG TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT

18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT  
CGAAAGTATG GTCGAAAACG AACTAAGGTA ACCACTATCT TGGTCCATGA

18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTTAGAATT  
AAAGATACAC CTTAGTCCGA CAACTGTCGA TACTAGGTCT ACAATCTTAA

18701 ATTGAAAATC ATGGAAGTGA AGATGAACTT CCAAATTACT GCTTTCCACT  
TAACTTTTAG TACCTTGACT TCTACTTGAA GGTTTAATGA CGAAAGGTGA

18751 GGGAGGTGTG ATTAATACAG AGACTCTTAC CAAGGTAAAA CCTAAAAACG  
CCCTCCACAC TAATTATGTC TCTGAGAATG GTTCCATTTT GGATTTTGTC

18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAATTTTC AGATAAAAAAT  
CAGTCCTTTT ACCTACCTTT TTTCTACGAT GTCTTAAAAG TCTATTTTTA

18851 GAAATAAGAG TTGGAAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA  
CTTTATTCTC AACCTTTATT AAAACGGTAC CTTTAGTTAG ATTTACGGTT

Figure 27T



18951 AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC  
TCGATTTCAT GTCAGGAAGG TTGCATTTTT AAAGACTATT GGGTTTGTGG

19001 TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA  
ATGCTGATGT ACTTGTTCGC TCACCACCGA GGGCCCGATC ACCTGACGAT

19051 CATTAACTTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC  
GTAATTGGAA CCTCGTGCGA CCAGGGAAC TATATACCTG TTGCAGTTGG

19101 CATTTAACCA CCACCGCAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG  
GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC

19151 GGCAATGGTC GCTATGTGCC CTTCCACATC CAGGTGCCTC AGAAGTTCTT  
CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA

19201 TGCCATTAAA AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGGA  
ACGGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT

19251 ACTTCAGGAA GGATGTTAAC ATGGTTCTGC AGAGCTCCCT AGGAAATGAC  
TGAAGTCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG

19301 CTAAGGGTTG ACGGAGCCAG CATTAAGTTT GATAGCATTT GCCTTTACGC  
GATTCCCAAC TGCTTCGGTC GTAATTCAAA CTATCGTAAA CGGAAATGCG

19351 CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC  
GTGGAAGAAG GGTACCGGG TGTGTGGCG GAGGTGCGAA CTCCGGTACG

19401 TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC  
AATCTTTGCT GTGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGCGG

19451 AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT  
TTGTACGAGA TGGGATATGG GCGGTTGCGA TGGTTGCACG GGTATAGGTA

19501 CCCCTCCCGC AACTGGGCGG CTTTCCGCGG CTGGGCCTTC ACGCGCCTTA  
GGGGAGGGCG TTGACCCGCC GAAAGGCGCC GACCCGGAAG TGCGCGGAAT

19551 AGACTAAGGA AACCCCATCA CTGGGCTCGG GCTACGACCC TTATTACACC  
TCTGATTCTT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG

19601 TACTCTGGCT CTATACCCTA CCTAGATGGA ACCTTTTACC TCAACCACAC  
ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG

19651 CTTTAAGAAG GTGGCCATTA CCTTTGACTC TTCTGTCAGC TGGCCTGGCA  
GAAATTCTTC CACCGGTAAT GGAACTGAG AAGACAGTCG ACCGGACCGT

19701 ATGACCGCCT GCTTACCCCC AACGAGTTTG AAATTAAGCG CTCAGTTGAC  
TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTTAATTGCG GAGTCAACTG

19751 GGGGAGGGTT ACAACGTTGC CCAGTGTAAC ATGACCAAAG ACTGGTTCTT  
CCCCTCCCAA TGTGCAACG GGTACATTG TACTGGTTTC TGACCAAGGA

19801 GGTAACAATG CTAGCTAACT ATAACATTGG CTACCAGGGC TTCTATATCC  
CCATGTTTAC GATCGATTGA TATTGTAACC GATGGTCCCG AAGATATAGG

Figure 274

19851 CAGAGAGCTA CAAGGACCGC ATGTACTCCT TCTTTAGAAA CTTCAGCCC  
GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTCGGG

19901 ATGAGCCGTC AGGTGGTGA TGATACTAAA TACAAGGACT ACCAACAGGT  
TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCTGA TGTTGTCCA

19951 GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTTGGC TACCTTGCCC  
CCCGTAGGAT GTGGTTGTGT TGTGAGACC TAAACAACCG ATGGAACGGG

20001 CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACCTCCC CTATCCGCTT  
GGTGGTACGC GCTTCCTGTC CGGATGGGAC GATTGAAGGG GATAGGCGAA

20051 ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTCTTTGCGA  
TATCCGTCTT GCGTCAACT GTCGTAATGG GTCTTTTCA AAGAAACGCT

20101 TCGCACCTTT TGGCGCATCC CATTCTCCAG TAACTTTATG TCCATGGGGC  
AGCGTGGGAA ACCGCGTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCGC

20151 CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAACTC CGCCACGCG  
GTGAGTGTCT GGACCCGGTT TTGGAAGAGA TGCGGTTGAG GCGGGTGC

20201 CTAGACATGA CTTTTGAGGT GGATCCCATG GACGAGCCCA CCCTTCTTTA  
GATCTGTACT GAAAACTCCA CCTAGGGTAC CTGCTCGGGT GGAAGAAAT

20251 TGTTTTGTTT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACCGCG  
ACAAAACAAA CTTCAGAAAC TGCACCAGGC ACACGTGGTC GCGGTGGCGC

20301 GCGTCATCGA AACCGTGTAC CTGCGCACGC CCTTCTCGGC CGGCAACGCC  
CGCAGTAGCT TTGGCACATG GACGCGTGCG GGAAGAGCCG GCCGTTGCGG

20351 ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC  
TGTTGTATTT CTTCGTTTCGT TGTAGTTGTT GTCGACGGCG GTACCCGAGG

20401 AGTGAGCAGG AACTGAAAGC CATTGTCAA GATCTTGGTT GTGGGCCATA  
TCACTCGTCC TTGACTTTTCG GTAACAGTTT CTAGAACCAA CACCCGGTAT

20451 TTTTTTGGGC ACCTATGACA AGCGCTTTCC AGGCTTTGTT TCTCCACACA  
AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501 AGCTCGCCTG CGCCATAGTC AATACGGCCG GTCGCGAGAC TGGGGGCGTA  
TCGACCGGAC GCGGTATCAG TTATGCCGGC CAGCGCTCTG ACCCCCGCAT

20551 CACTGGATGG CCTTGCCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT  
GTGACCTACC GGAAACGGAC CTTGGGCGTG AGTTTTTGTA CGATGGAGAA

20601 TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTTG  
ACTCGGGAAC CCGAAAAGAC TGGTCGCTGA GTTCGTCAA ATGGTCAAAC

20651 AGTACGAGTC ACTCCTGCGC CGTAGCGCCA TTGCTTCTTC CCCCACCGC  
TCATGCTCAG TGAGGACGCG GCATCGCGGT AACGAAGAAG GGGGCTGGCG

20701 TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC  
ACATATTGCG ACCTTTTCAG GTGGGTTTCG CATGTCCCCG GGTTGAGCCG

20751 CGCCTGTGGA CTATTCTGCT GCATGTTTCT CCACGCCTTT GCCAACTGGC  
GCGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGGTTGACCG

Figure 27 V.

20851 CCCAACTCCA TGCTCAACAG TCCCCAGGTA CAGCCCACCC TCGTTCGCAA  
GGGTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT

20901 CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA  
GGTCCTTGTC GAGATGTCGA AGGACCTCGC GGTGAGCGGG ATGAAGGCGT

20951 GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTGTCA CTGAAAAAC  
CGGTGTCACG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG

21001 ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA  
TACATTTTTA TTACATGATC TCTGTGAAAG TTATTTCCGT TTACGAAAAAT

21051 TTTGTACACT CTCGGGTGAT TATTTACCCC CACCCTTGCC GTCTGCGCCG  
AAACATGTGA GAGCCCCTA ATAAATGGGG GTGGGAACGG CAGACGCGGC

21101 TTAAAAATC AAAGGGGTTT TGCCGCGCAT CGCTATGCGC CACTGGCAGG  
AAATTTTTAG TTTCCCAAG ACGGCGCGTA GCGATACGCG GTGACCGTCC

21151 GACACGTTGC GATACTGGTG TTAGTGCTC CACTTAACT CAGGCACAAC  
CTGTGCAACG CTATGACCAC AAATCACGAG GTGAATTTGA GTCCGTGTTG

21201 CATCCGCGGC AGCTCGGTGA AGTTTTCACT CCACAGGCTG CGCACCATCA  
GTAGGCGCCG TCGAGCCACT TCAAAAGTGA GGTGTCCGAC GCGTGGTAGT

21251 CCAACGCGTT TAGCAGGTCG GCGCCGATA TCTTGAAGTC GCAGTTGGGG  
GGTTGCGCAA ATCGTCCAGC CCGCGGCTAT AGAACTTCAG CGTCAACCCC

21301 CCTCCGCCCT GCGCGCGCGA GTTGCGATAC ACAGGGTTGC AGCACTGGAA  
GGAGGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT

21351 CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCACGCTC TTGTCGGAGA  
GTGATAGTCG CGGCCACCA CGTGCGACCG GTCGTGCGAG AACAGCCTCT

21401 TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC  
AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCGCTT GCCTCAGTTG

21451 TTTGGTAGCT GCCTTCCCAA AAAGGGCGCG TGCCAGGCT TTGAGTTGCA  
AAACCATCGA CGGAAGGGTT TTTCCCGCGC ACGGGTCCGA AACTCAACGT

21501 CTCGCACCGT AGTGGCATCA AAAGGTGACC GTGCCCAGTC TGGGCGTTAG  
GAGCGTGGCA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC

21551 GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAGC CACCTGAGCC  
CTATGTCGCG GACGTATTTT CGGAACTAGA CGAATTTTCG GTGGACTCGG

21601 TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAACTGATT  
AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGGCC TTTTGACTAA

21651 GGCCGGACAG GCCGCGTCGT GCACGCAGCA CCTTCCGTCG GTGTGGAGA  
CCGGCCTGTC CGGCGCAGCA CGTCCGTCGT GGAACGCAGC CACAACCTCT

21701 TCTGCACCAC ATTTCCGGCC CACCGGTTCT TCACGATCTT GGCCTTGCTA  
AGACGTGGTG TAAAGCCGGG GTGGCCAAGA AGTGCTAGAA CCGGAACGAT

Figure 27 W

21801 AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT  
 TTAGTGACAG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCGA  
 21851 CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCGTGGGC  
 GCGGAAGCTA GAGTCGCGTC GCCACGTCGG TGTTCGCGCT CGGGCACCCG  
 21901 TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCTGCAG  
 AGCACTACGA ACATCCAGTG GAGACGTTTG CTGACGTCCA TGCGGACGTC  
 21951 GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGGTCAGCT  
 CTTAGCGGGG TAGTAGCAGT GTTTCAGAA CAACGACCAC TTCCAGTCGA  
 22001 GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGACATAC GGCCGCCAGA  
 CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGGCGGTCT  
 22051 GCTTCCACTT GGTCAGGCAG TAGTTTGAAG TTCGCCTTTA GATCGTTATC  
 CGAAGGTGAA CCAGTCCGTC ATCAAACCTC AAGCGGAAAT CTAGCAATAG  
 22101 CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC  
 GTGCACCATG AACAGSTAGT CCGCGCGCGC TCGGAGGTAC GGAAGAGGG  
 22151 ACGCAGACAC GATCGGCACA CTCAGCGGGT TCATCACCGT AATTTCACTT  
 TCGCTCTGTG CTAGCCGTGT GAGTCGCCCC AGTAGTGGA TTAAGTGAA  
 22201 TCCGCTTCGC TGGGCTCTTC CTCTTCTCT TCGCTCCGA TACCACGCGC  
 AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTGCGCG  
 22251 CACTGGGTCG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTGC  
 GTGACCCAGC AGAAGTAAGT CCGCGGCGTG ACACGCGAAT GGAGGAAACG  
 22301 CATGCTTGAT TAGCACCAGT GGGTTGCTGA AACCACCAT TTGTAGCGCC  
 GTACGAATA ATCGTGGA CCAACGACT TTGGGTGGTA AACATCGCGG  
 22351 ACATCTTCTC TTTCTTCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG  
 TGTAGAAGAG AAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC  
 22401 GCGCTCGGGC TTGGGAGAAG GCGCTTCTT TTTCTTCTTG GCGCAATGG  
 CGCGAGCCCG AACCTCTTC CCGGAAGAA AAAGAAGAAC CCGCGTTACC  
 22451 CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC  
 GGTTTAGGCG GCGGCTCCAG CTACCGGCGC CCGACCCACA CGCGCCGTGG  
 22501 AGCGCGTCTT GTGATGAGTC TTCCTCGTCC TCGGACTCGA TACGCCGCTT  
 TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCCTGAGCT ATGCGGCGGA  
 22551 CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGGCGGCGAC GGGGACGGGG  
 GTAGGCGAAA AAACCCCGC GGGCCCTCC GCCGCGCTG CCCCTGCCCC  
 22601 ACGACACGTC CTCCATGGTT GGGGACGTC GCGCCGACC GCGTCCGCGC  
 TGCTGTGCAG GAGGTACCAA CCCCCTGCAG CGCGGCGTGG CGCAGGCGCG  
 22651 TCGGGGGTGG TTTGCGGCTG CTCCTCTTCC CGACTGGCCA TTTCTTCTC  
 AGCCCCACC AAAGCGCGAC GAGGAGAAGG GCTGACCGGT AAAGGAAGAG

Figure 27X

22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG  
GGCGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC

22801 CCTACCACCT TCCCCGTCGA GGCACCCCCG CTTGAGGAGG AGGAAGTGTAT  
GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTCACTA

22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG  
ATAGCTCGTC CTGGGTCCAA AACATTGCT TCTGCTGCTC CTGGCGAGTC

22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAAACGAG  
ATGGTTGTCT CCTATTTTTC GTTCTGGTCC TGTTGCGTCT CCGTTTGCTC

22951 GAACAAGTCG GGCGGGGGGA CGAAAGGCAT GCGGACTACC TAGATGTGGG  
CTTGTTTCAGC CCGCCCCCCT GCTTTCGTA CCGCTGATGG ATCTACACCC

23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG  
TCTGCTGCAC GACAACCTCG TAGACGTCGC GGTACGCGG TAATAGACGC

23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC  
TGCGCAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG

23101 CTTGCCTACG AACGCCACCT ATTCTCACCG CGCGTACCCC CCAAACGCCA  
GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTGCGGT

23151 AGAAAACGGC ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT  
TCTTTTGCCG TGTACGCTCG GGTGCGCGC GGAGTTGAAG ATGGGGCATA

23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTT CCAAACTGC  
AACGGCACGG TCTCCACGAA CCGTGGATAG TGTAAGAAAA GGTGTTGACG

23251 AAGATACCCC TATCCTGCCG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT  
TTCATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC TGTTGCTCGA

23301 GGCCTTGCGG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG  
CCGGAACGCC GTCCCGGAC AGTATGGACT ATAGCGGAGC GAGTTGCTTC

23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAC  
ACGGTTTTTA GAAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCCGTTTG

23401 GCTCTGCAAC AGGAAAACAG CGAAAATGAA AGTCACTCTG GAGTGTGGT  
CGAGACGTTG TCCTTTTCTC GCTTTTACTT TCAGTGAGAC CTCACAACCA

23451 GGAACCTCGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG  
CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC

23501 AGGTCACCCA CTTTGCTTAC CCGGCACTTA ACCTACCCCC CAAGGTCATG  
TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC

23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCCTGGAGAG  
TCGTGTCAGT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGGACCTCTC

23601 GGATGCAAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCAGTTGGCG  
CCTACGTTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC

Figure 27 Y

23701 GAGCGACGCA AACTAATGAT GGCCGCACTG CTCGTTACCG TGGAGCTTGA  
CTCGCTGCGT TTGATTACTA CCGGCGTCAC GAGCAATGGC ACCTCGAACT

23751 GTGCATGCAG CGGTTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG  
CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTCGCG TTCGATCTCC

23801 AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCCTGCAAG  
TTTGTAACGT GATGTGAAA GCTGTCCCGA TGCATGCGGT CCGGACGTTT

23851 ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA  
TAGAGGTTGC ACCTCGAGAC GTTGGACCAG AGGATGGAAC CTTAAAACGT

23901 CGAAAACCGC CTTGGGCAAA ACGTGCTTCA TTCCACGCTC AAGGGCGAGG  
GCTTTTGGCG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCCTCC

23951 CGCGCCGCGA CTACGTCCGC GACTGCGTTT ACTTATTTCT ATGCTACACC  
GCGCGGCGCT GATGCAGGCG CTGACGCAA TGAATAAAGA TACGATGTGG

24001 TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGGAGG AGTGCAACCT  
ACCGTCTGCC GGTACCCGCA AACCGTCGTC ACGAACCTCC TCACGTTGGA

24051 CAAGGAGCTG CAGAACTGC TAAAGCAAAA CTTGAAGGAC CTATGGACGG  
GTTCTCGAC GTCTTTGACG ATTTCGTTTT GAACCTCCTG GATACCTGCC

24101 CCTTCAACGA GCGCTCCGTG GCCGCGCACC TGGCGGACAT CATTTTCCCC  
GGAAGTTGCT CGCGAGGCAC CGGCGCGTGG ACCGCCTGTA GTAAAAGGGG

24151 GAACGCCTGC TTAACCCTT GCAACAGGGT CTGCCAGACT TCACCAGTCA  
CTTGCGGACG AATTTTGGGA CGTTGTCCCA GACGGTCTGA AGTGGTCAGT

24201 AAGCATGTTG CAGAACTTTA GGAACCTTAT CCTAGAGCGC TCAGGAATCT  
TTCGTACAAC GTCTTGAAAT CCTTGAAATA GGATCTCGCG AGTCCTTAGA

24251 TGCCCGCCAC CTGCTGTGCA CTTCTAGCG ACTTTGTGCC CATTAAGTAC  
ACGGGCGGTG GACGACACGT GAAGGATCGC TGAAACACGG GTAATTCATG

24301 CGCGAATGCC CTCCGCCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC  
GCGCTTACGG GAGGCGGCGA AACCCCGGTG ACGATGGAAG ACGTCGATCG

24351 CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG  
GTTGATGGAA CGGATGGTGA GACTGTATTA CCTTCTGCAC TCGCCACTGC

24401 GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC  
CAGATGACCT CACAGTGACA GCGACGTTGG ATACGTGGGG CGTGGCGAGG

24451 CTGGTTTGCA ATTGCGAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT  
GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAT AGCCATGGAA

24501 TGAGCTGCAG GGTCCCTCGC CTGACGAAAA GTCCGCGGCT CCGGGGTTGA  
ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCCAACT

24551 AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT  
TTGAGTGAGG CCCCAGACCC TGCAGCCGAA TGGAAGCGTT TAAACATGGA

Figure 272

24601 GAGGACTACC ACGCCACGA GATTAGGTTT TACGAAGACC AATCCCGCCC  
CTCCTGATGG TCGGGTGCT CTAATCCAAG ATGCTTCTGG TTAGGGCGGG

24651 GCCTAATGCG GAGCTTACCG CCTGCGTCAT TACCCAGGGC CACATTCTTG  
CGGATTACGC CTCGAATGGC GGACGCAGTA ATGGGTCCCG GTGTAAGAAC

24701 GCCAATTGCA AGCCATCAAC AAAGCCCGCC AAGAGTTTCT GCTACGAAAG  
CGGTAAACGT TCGGTAGTTG TTTCGGGCGG TTCTCAAAGA CGATGCTTTC

24751 GGACGGGGGG TTTACTTGGA CCCCCAGTCC GGCAGGAGC TCAACCCAAT  
CCTGCCCCC AAATGAACCT GGGGGTCAGG CCGCTCCTCG AGTTGGGTGA

24801 CCCCCGCGG CCGCAGCCCT ATCAGCAGCA GCCGCGGGCC CTTGCTTCCC  
GGGGGCGGG GCGTCGCGA TAGTCGTCGT CCGCGCCCGG GAACGAAGGG

24851 AGGATGGCAC CAAAAAGAA GCTGCAGCTG CCGCCGCCAC CCACGGACGA  
TCCTACCGTG GGTTTTCTT CGACGTCGAC GCGGCGGGTG GGTGCCTGCT

24901 GGAGGAATAC TGGGACAGTC AGGCAGAGGA GGTTTTGAC GAGGAGGAGG  
CCTCCTTATG ACCCTGTCAG TCCGTCTCCT CCAAACCTG CTCCTCTCC

24951 AGGACATGAT GGAAGACTGG GAGAGCCTAG ACGAGGAAGC TTCCGAGGTC  
TCCTGTACTA CCTCTGACC CTCTCGGATC TGCTCCTTCG AAGGCTCCAG

25001 GAAGAGGTGT CAGACGAAAC ACCGTCACCC TCGGTCGCAT TCCCCTCGCC  
CTTCTCCACA GTCTGCTTTG TGGCAGTGGG AGCCAGCGTA AGGGGAGCGG

25051 GGCGCCCCAG AAATCGGCA CCGGTTCCAG CATGGCTACA ACCTCCGCTC  
CCGCGGGGTC TTTAGCCGTT GGCCAAGGTC GTACCGATGT TGGAGGCGAG

25101 CTCAGGCGCC GCCGGCACTG CCCGTTCCGC GACCCAACCG TAGATGGGAC  
GAGTCCGCGG CCGCCGTGAC GGGCAAGCGG CTGGGTTGGC ATCTACCCTG

25151 ACCACTGGAA CCAGGGCCGG TAAGTCCAAG CAGCCGCCGC CGTTAGCCCA  
TGGTGACCTT GTCCCGGCC ATTCAGGTTT GTCGGCGGCG GCAATCGGGT

25201 AGAGCAACAA CAGCGCCAAG GCTACCGCTC ATGGCGCGGG CACAAGAAGC  
TCTCGTTGTT GTCGCGGTTT CGATGGCGAG TACCGCGCCC GTGTTCTTGC

25251 CCATAGTTGC TTGCTTGCAA GACTGTGGGG GCAACATCTC CTTGCCCCG  
GGTATCAACG AACGAACGTT CTGACACCCC CGTTGTAGAG GAAGCGGGCG

25301 CGCTTTCTTC TCTACCATCA CGGCGTGGCC TTCCCCGTA ACATCCTGCA  
GCGAAAGAAG AGATGGTAGT GCCGACCCG AAGGGGGCAT TGTAGGACGT

25351 TTACTACCGT CATCTCTACA GCCCATACTG CACCGGCGGC AGCGGCAGCA  
AATGATGGCA GTAGAGATGT CGGGTATGAC GTGGCCGCCG TCGCCGTCGT

25401 ACAGCAGCGG CCACACAGAA GCAAAGGCGA CCGGATAGCA AGACTCTGAC  
TGTCGTCGCC GGTGTGTCTT CGTTTCCGCT GGCCTATCGT TCTGAGACTG

25451 AAAGCCCAAG AAATCCACAG CGGCGGCAGC AGCAGGAGGA GGAGCGCTGC  
TTTCGGGTTT TTTAGGTGTC GCCGCCGTCG TCGTCCTCCT CCTCGCGACG

25501 GTCTGGCGCC CAACGAACCC GTATCGACCC GCGAGCTTAG AACAGGATT  
CAGACCGCGG GTTGCTTGGG CATAGCTGGG CGCTCGAATC TTTGTCCTAA

Figure 27 AA

25551 TTTCCCACTC TGTATGCTAT ATTTCAACAG AGCAGGGGCC AAGAAACAAGA  
AAAGGGTGAG ACATACGATA TAAAGTTGTC TCGTCCCCGG TTCTTGTTCT

25601 GCTGAAAATA AAAAACAGGT CTCTGCGATC CCTCACCCGC AGCTGCCTGT  
CGACTTTTAT TTTTGTCCA GAGACGCTAG GGAGTGGGCG TCGACGGACA

25651 ATCACAAAAG CGAAGATCAG CTTGCGCGCA CGCTGGAAGA CGCGGAGGCT  
TAGTGTTTTTC GCTTCTAGTC GAAGCCGCGT GCGACCTTCT GCGCCTCCGA

25701 CTCTTCAGTA AATACTGCGC GCTGACTCTT AAGGACTAGT TTCGCGCCCT  
GAGAAGTCAT TTATGACGCG CGACTGAGAA TTCCTGATCA AAGCGCGGGA

25751 TTCTCAAATT TAAGCGCGAA AACTACGTCA TCTCCAGCGG CCACACCCGG  
AAGAGTTTAA ATTGCGCGTT TTGATGCAGT AGAGGTGCGC GGTGTGGGCC

25801 CGCCAGCACC TGTGTGCAGC GCCATTATGA GCAAGGAAAT TCCCACGCCC  
GCGGTCGTGG ACAACAGTCG CGGTAATACT CGTTCCTTTA AGGGTGCGGG

25851 TACATGTGGA GTTACCAGCC ACAAATGGGA CTTGCGGCTG GAGCTGCCCC  
ATGTACACCT CAATGGTTCG TGTTTACCTT GAACCCGAC CTCGACGGGT

25901 AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC CACATGATAT  
TCTGATGAGT TGGGCTTATT TGATGTACTC GCGCCCTGGG GTGTACTATA

25951 CCCGGGTCAA CGGAATACGC GCCCACCAGAA ACCGAATTCT CCTGGAACAG  
GGGCCAGTT GCCTTATGCG CGGGTGGCTT TGGCTTAAGA GGACCTTGTC

26001 GCGGCTATTA CCACCACACC TCGTAATAAC CTTAATCCCC GTAGTTGGCC  
CGCCGATAAT GGTGGTGTGG AGCATTATTG GAATTAGGGG CATCAACCGG

26051 CGCTGCCCTG GTGTACCAGG AAAGTCCCGC TCCCACCACT GTGGTACTTC  
GCGACGGGAC CACATGGTCC TTTCAGGGCG AGGGTGGTGA CACCATGAAG

26101 CCAGAGACGC CCAGGCCGAA GTTCAGATGA CTAAGTCAGG GGCGCAGCTT  
GGTCTCTGCG GGTCCGGCTT CAAGTCTACT GATTGAGTCC CCGCGTCGAA

26151 GCGGGCGGCT TTCGTACAG GGTGCGGTCG CCCGGGCAGG GTATAACTCA  
CGCCCGCCGA AAGCAGTGTC CCACGCCAGC GGGCCCGTCC CATATTGAGT

26201 CCTGACAATC AGAGGGCGAG GTATTCAGCT CAACGACGAG TCGGTGAGCT  
GGACTGTTAG TCTCCGCTC CATAAGTCGA GTTGCTGCTC AGCCACTCGA

26251 CCTCGCTTGG TCTCCGTCCG GACGGGACAT TTCAGATCGG CGGCGCCGGC  
GGAGCGAACC AGAGGCAGGC CTGCCCTGTA AAGTCTAGCC GCCGCGGCCG

26301 CGCTCTTCAT TCACGCCTCG TCAGGCAATC CTAAGTCTGC AGACCTCGTC  
GCGAGAAGTA AGTGCGGAGC AGTCCGTTAG GATTGAGACG TCTGGAGCAG

26351 CTCTGAGCCG CGCTCTGGAG GCATTGGAAC TCTGCAATTT ATTGAGGAGT  
GAGACTCGGC GCGAGACCTC CGTAACCTTG AGACGTAAA TAACTCCTCA

26401 TTGTGCCATC GGTCTACTTT AACCCTTCT CGGGACCTCC CGGCCACTAT  
AACACGGTAG CCAGATGAAA TTGGGGAAGA GCCCTGAGG GCCGGTGATA

26451 CCGGATCAAT TTATTCTTAA CTTTGACGCG GTAAAGGACT CGGCGGACGG  
GGCCTAGTTA AATAAGGATT GAAACTGCGC CATTTCCTGA GCCGCTGCC

Figure 27 AB



26501 CTACGACTGA ATGTTAAGTG GAGAGGCAGA GCAACTGCGC CTGAAACACC  
GATGCTGACT TACAATTACAC CTCTCCGTCT CGTTGACGCG GACTTTGTGG

26551 TGGTCCACTG TCGCCGCCAC AAGTGCTTTG CCCGCGACTC CGGTGAGTTT  
ACCAGGTGAC AGCGGCGGTG TTCACGAAAC GGGCGCTGAG GCCACTCAAA

26601 TGCTACTTTG AATTGCCCGA GGATCATATC GAGGGCCCGG CGCACGGCGT  
ACGATGAAAC TTAACGGGCT CCTAGTATAG CTCCCGGGCC GCGTGCCGCA

26651 CCGGCTTACC GCCCAGGGAG AGCTTGCCCC TAGCCTGATT CGGGAGTTTA  
GGCCGAATGG CGGGTCCCTC TCGAACGGGC ATCGGACTAA GCCCTCAAAT

26701 CCCAGCGCCC CCTGCTAGTT GAGCGGGACA GGGGACCCTG TGTTCCTACT  
GGGTCGCGGG GGACGATCAA CTCGCCCTGT CCCCTGGGAC ACAAGAGTGA

26751 GTGATTGCA ACTGTCTTAA CCCTGGATTA CATCAAGATC TTTGTTGCCA  
CACTAAACGT TGACAGGATT GGGACCTAAT GTAGTTCTAG AAACAACGGT

26801 TCTCTGTGCT GAGTATAATA AATACAGAAA TTAAAATATA CTGGGGCTCC  
AGAGACACGA CTCATATTAT TTATGTCTTT AATTTTATAT GACCCCGAGG

26851 TATCGCCATC CTGTAAACGC CACCGTCTTC ACCCGCCCAA GCAAACCAAG  
ATAGCGGTAG GACATTTGCG GTGGCAGAAG TGGCGGGTT CGTTTGTTTC

26901 GCGAACCTTA CCTGGTACTT TTAACATCTC TCCCTCTGTG ATTTACAACA  
CGCTTGGAAT GGACCATGAA AATTGTAGAG AGGGAGACAC TAAATGTTGT

26951 GTTTCAACCC AGACGGAGTG AGTCTACGAG AGAACCTCTC CGAGCTCAGC  
CAAAGTTGGG TCTGCCTCAC TCAGATGCTC TCTTGAGAG GCTCGAGTCG

27001 TACTCCATCA GAAAAACAC CACCCTCCTT ACCTGCCGGG AACGTACGAG  
ATGAGGTAGT CTTTTTTGTG GTGGGAGGAA TGGACGGCCC TTGCATGCTC

27051 TGCGTCACCG GCCGCTGCAC CACACCTACC GCCTGACCGT AAACCAGACT  
ACGCAGTGGC CGGCGACGTG GTGTGGATGG CGGACTGGCA TTTGGTCTGA

27101 TTTTCCGGAC AGACCTCAAT AACTCTGTTT ACCAGAACAG GAGGTGAGCT  
AAAAGGCCTG TCTGGAGTTA TTGAGACAAA TGGTCTTGTC CTCCACTCGA

27151 TAGAAAACCC TTAGGGTATT AGGCCAAAGG CGCAGCTACT GTGGGGTTTA  
ATCTTTTGGG AATCCCATAA TCCGGTTTCC GCGTCGATGA CACCCCAAAT

27201 TGAACAATTC AAGCAACTCT ACGGGCTATT CTAATTCAGG TTTCTCTAGA  
ACTTGTTAAG TTCGTTGAGA TGCCCCGATAA GATTAAGTCC AAAGAGATCT

27251 ATCGGGGTTG GGGTTATTCT CTGTCTTGTTG ATTCTCTTTA TTCTTATACT  
TAGCCCCAAC CCCAATAAGA GACAGAACAC TAAGAGAAAT AAGAATATGA

27301 AACGCTTCTC TGCCTAAGGC TCGCCGCCTG CTGTGTGCAC ATTTGCATTT  
TTGCGAAGAG ACGGATTCCG AGCGGCGGAC GACACACGTG TAAACGTAAA

27351 ATTGTCAGCT TTTTAAACGC TGGGGTCGCC ACCCAAGATG ATTAGGTACA  
TAACAGTCGA AAAATTTGCG ACCCCAGCGG TGGGTCTAC TAATCCATGT

27401 TAATCCTAGG TTTACTCACC CTTGCGTCAG CCCACGGTAC CACCCAAAAG  
ATTAGGATCC AAATGAGTGG GAACGCAGTC GGGTGCCATG GTGGGTTTTT

Figure 27AC

27451 GTGGATTTTA AGGAGCCAGC CTGTAATGTT ACATTTCGAG CTGAAGCTAA  
CACCTAAAT TCCTCGGTCG GACATTACAA TGTAAGCGTC GACTTCGATT

27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA  
ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGACTT TTCGACGAAT

27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG  
AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC

27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA  
GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTCAGT

27651 TAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA  
ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT

27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAAA TTGTGTGGAA  
ACATGTACTC GTTGTGCATA TTCAACACCG GGGGTGTTTT AACACACCTT

27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT  
TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA

27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG  
CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC

27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC  
TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG

27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT  
ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA

27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCTGCTC AATACCATTG  
TTAATCTTAT CCTAAATTTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG

28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA  
GGGACTTGTT AACTGAGATA CACCTATAC GAGGTGCGGA TGTTGGAAT

28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG  
TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC

28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA  
GCCTAAACAA GGTGAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT

28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA  
TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT

28201 CCCCAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG  
GGGGTTCAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC

28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT  
CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA

28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT  
CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGATAGATATC AGGGTAGTAA

28351 GTGCTACACC CAAACAATGA TGGAATCCAT AGATTGGACG GACTGAAACA  
CAGGATGTGG GTTGTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

28451 TTTTATATTA CTGACCCTTG TTGCGCTTTT TTGTGCGTGC TCCACATTGG  
AAAATATAAT GACTGGGAAC AACGCGAAAA AACACGCACG AGGTGTAACC

28501 CTGCGGTTTC TCACATCGAA GTAGACTGCA TTCCAGCCTT CACAGTCTAT  
GACGCCAAAG AGTGTAGCTT CATCTGACGT AAGGTCGGAA GTGTCAGATA

28551 TTGCTTTACG GATTTGTCAC CCTCACGCTC ATCTGCAGCC TCATCACTGT  
AACGAAATGC CTAAACAGTG GGAGTGCAG TAGACGTCGG AGTAGTGACA

28601 GGTCATCGCC TTTATCCAGT GCATTGACTG GGTCTGTGTG CGCTTTGCAT  
CCAGTAGCGG AAATAGGTCA CGTAACTGAC CCAGACACAC GCGAAACGTA

28651 ATCTCAGACA CCATCCCCAG TACAGGGACA GGACTATAGC TGAGCTTCTT  
TAGAGTCTGT GGTAGGGGTC ATGTCCCTGT CCTGATATCG ACTCGAAGAA

28701 AGAATTCTTT AATTATGAAA TTTACTGTGA CTTTTCTGCT GATTATTTGC  
TCTTAAGAAA TTAATACTTT AAATGACACT GAAAAGACGA CTAATAAACG

28751 ACCCTATCTG CGTTTTGTTC CCCGACCTCC AAGCCTCAAA GACATATATC  
TGGGATAGAC GCAAAACAAG GGGCTGGAGG TTCGGAGTTT CTGTATATAG

28801 ATGCAGATTC ACTCGTATAT GGAATATTCC AAGTTGCTAC AATGAAAAAA  
TACGTCTAAG TGAGCATATA CCTTATAAGG TTCAACGATG TTACTTTTTT

28851 GCGATCTTTC CGAAGCCTGG TTATATGCAA TCATCTCTGT TATGGTGTTC  
CGCTAGAAAG GCTTCGGACC AATATACGTT AGTAGAGACA ATACCACAAG

28901 TGCAGTACCA TCTTAGCCCT AGCTATATAT CCCTACCTTG ACATTGGCTG  
ACGTCATGGT AGAATCGGGA TCGATATATA GGGATGGAAC TGTAACCGAC

28951 GAACGCAATA GATGCCATGA ACCACCCAAC TTTCCCCGCG CCCGCTATGC  
CTTGCGTTAT CTACGGTACT TGGTGGGTTG AAAGGGGCGC GGGCGATACG

29001 TTCCACTGCA ACAAGTTGTT GCCGGCGGCT TTGTCCCAGC CAATCAGCCT  
AAGGTGACGT TGTTCAACAA CGGCCGCCGA AACAGGGTCG GTTAGTCGGA

29051 CGCCCACCTT CTCCCACCCC CACTGAAATC AGCTACTTTA ATCTAACAGG  
GCGGGTGGAA GAGGGTGGGG GTGACTTTAG TCGATGAAAT TAGATTGTCC

29101 AGGAGATGAC TGACACCCTA GATCTAGAAA TGGACGGAAT TATTACAGAG  
TCCTCTACTG ACTGTGGGAT CTAGATCTTT ACCTGCCTTA ATAATGTCTC

29151 CAGCGCCTGC TAGAAAGACG CAGGGCAGCG GCCGAGCAAC AGCGCATGAA  
GTCGCGGACG ATCTTTCTGC GTCCCGTCGC CGGCTCGTTG TCGCGTACTT

29201 TCAAGAGCTC CAAGACATGG TTAACCTGCA CCAGTGCAAA AGGGGTATCT  
AGTTCTCGAG GTTCTGTACC AATTGAACGT GGTCACGTTT TCCCCATAGA

29251 TTTGTCTCGT AAAGCAGGCC AAAGTCACCT ACGACAGTAA TACCACCGGA  
AAACAGAGCA TTTCGTCCGG TTTCAGTGGA TGCTGTCATT ATGGTGGCCT

29301 CACCGCCTTA GCTACAAGTT GCCAACCAAG CGTCAGAAAT TGGTGGTCAT  
GTGGCGGAAT CGATGTTCAA CGGTGGTTC GCAGTCTTTA ACCACCAGTA

Figure 27AE

29401 GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCTTATT  
CGACGTAAGT GAGTGGAACA GTTCCTGGAC TCCTAGAGAC GTGGGAATAA

29451 AAGACCCCTGT GCGGTCTCAA AGATCTTATT CCCTTTAACT AATAAAAAAA  
TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTPTT

29501 AATAATAAAG CATCACTTAC TTAAAATCAG TTAGCAAATT TCTGTCCAGT  
TTATTATTTT GTAGTGAATG AATTTTAGTC AATCGTTTAA AGACAGGTCA

29551 TTATTCAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT  
AATAAGTCGT CGTGGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA

29601 CCTCCTGGCT GCAAACCTTC TCCACAATCT AAATGGAATG TCAGTTTCCT  
GGAGGACCGA CGTTTGAAAG AGGTGTTAGA TTTACCTTAC AGTCAAAGGA

29651 CCTGTTCCCTG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG  
GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC

29701 CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC  
GCGCGTTCTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG

29751 GGAAACCGGT CCTCCAACCTG TGCCTTTTCT TACTCCTCCC TTTGTATCCC  
CCTTTGGCCA GGAGGTTGAC ACGGAAAAGA ATGAGGAGGG AACATAGGG

29801 CCAATGGGTT TCAAGAGAGT CCCCTGGGG TACTCTCTTT GCGCCTATCC  
GGTTACCCAA AGTTCTCTCA GGGGGACCCC ATGAGAGAAA CGCGGATAGG

29851 GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG  
CTTGAGATC AATGGAGGTT ACCGTACGAA CGCGAGTTTT ACCCGTTGCC

29901 CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCCAAAT GTAACCACTG  
GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGGTTTTA CATTGGTGAC

29951 TGAGCCCACC TCTCAAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT  
ACTCGGGTGG AGAGTTTTTT TGGTTCAGTT TGTATTTGGA CCTTTATAGA

30001 GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGACCC  
CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GGC GGCGTGG

30051 TCTAATGGTC GCGGGCAACA CACTCACCAT GCAATCACAG GCCCCGCTAA  
AGATTACCAG CGCCCGTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT

30101 CCGTGCACGA CTCCAAACTT AGCATTGCCA CCCAAGGACC CCTCACAGTG  
GGCACGTGCT GAGGTTTGAA TCGTAACGGT GGGTTCCTGG GGAGTGTAC

30151 TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGCCCCCTCA CCACCACCGA  
AGTCTTCCTT TCGATCGGGA CGTTTGTAGT CCGGGGGAGT GGTGGTGGCT

30201 TAGCAGTACC CTTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG  
ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC

30251 GTAGCTTGGG CATTGACTTG AAAGAGCCCA TTTATACACA AAATGGAAAA  
CATCGAACCC GTAACCTGAA TTTCTCGGGT AAATATGTGT TTTACCTTTT

Figure 27 AF

30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC  
AAACTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG

30401 AAACATAAGT TACTGGAGCC TTGGGTTTTG ATTCACAAGG CAATATGCAA  
TTTGATTTC AATGACCTCGG AACCCAAAAC TAAGTGTTCC GTTATACGTT

30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT  
GAATTACATC GTCCTCCTGA TTCCTAACTA AGAGTTTTGT CTGCGGAATA

30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC  
TGAACATCAA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG

30551 TAGGACAGGG CCCTCTTTTT ATAACTCAG CCCACAACCT GGATATTAAC  
ATCCTGTCCC GGGAGAAAAA TATTTGAGTC GGGTGTTGAA CCTATAATTG

30601 TACAACAAAG GCCTTTACTT GTTTACAGCT TCAAACAATT CCAAAAAGCT  
ATGTTGTTTC CGGAAATGAA CAAATGTGCA AGTTTGTTAA GGTTTTTCGA

30651 TGAGGTTAAC CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA  
ACTCCAATTG GATTCGTGAC GGTTCCTCAA CTACAACTG CGATGTCGGT

30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA  
ATCGGTAATT ACGTCCTCTA CCCGAACCTA AACCAAGTGG ATTACGTGGT

30751 AACACAAATC CCCTCAAAAC AAAAATTGGC CATGGCCTAG AATTTGATTG  
TTGTGTTTAG GGGAGTTTGT TTTTAAACCG GTACCGGATC TTAAACTAAG

30801 AAACAAGGCT ATGGTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA  
TTTGTTCCTA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAACGTGCTG

30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG  
GTCCACGGTA ATGTCATCCT TTGTTTTTAT TACTATTGCA TTGAAACACC

30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC  
TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTTACGTC TCTTTCTACG

30951 TAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAAATA CTGCTACAG  
ATTTGAGTGA AACCAGAATT GTTTTACACC GTCAGTTTAT GAACGATGTC

31001 TTTCAGTTTT GGCTGTAAA GGCAGTTTGG CTCCAATATC TGGAACAGTT  
AAAGTCAAAA CCGACAATTT CCGTCAAACC GAGGTATAG ACCTTGTCAA

31051 CAAAGTGCTC ATCTTATTAT AAGATTGAC GAAAATGGAG TGCTACTAAA  
GTTTCACGAG TAGAATAATA TTCTAACTG CTTTTACCTC ACGATGATTT

31101 CAATTCCTTC CTGGACCCAG AATATTGGAA CTTTAGAAAT GGAGATCTTA  
GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT

31151 CTGAAGGCAC AGCCTATACA AACGCTGTTG GATTTATGCC TAACCTATCA  
GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACGG ATTGGATAGT

31201 GCTTATCCAA AATCTCACGG TAAACTGCC AAAAGTAACA TTGTCAGTCA  
CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTCATTGT AACAGTCAGT

Figure 27 AG

31251 AGTTTACTTA AACGGAGACA AAACCTAAACC TGTAACACTA ACCATTACAC  
TCAAATGAAT TTGCCTCTGT TTTGATTTGG ACATTGTGAT TGGTAATGTG

31301 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG  
ATTTGCCATG TGTCCTTTGT CCTCTGTGTT GAGGTTACAG TATGAGATAC

31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTTGC  
AGTAAAAGTA CCTGACCAG ACCGGTGTG ATGTAATTAC TTTATAAACG

31401 CACATCCTCT TACACTTTTT CATACTTGC CCAAGAATAA AGAATCGTTT  
GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTCTTATT TCTTAGCAAA

31451 GTGTTATGTT TCAACGTGTT TATTTTTCAA TTGCAGAAAA TTTCAAGTCA  
CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT

31501 TTTTTCATTC AGTAGTATAG CCCCACCACC ACATAGCTTA TACAGATCAC  
AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG

31551 CGTACCTTAA TCAAACTCAC AGAACCCTAG TATTCAACCT GCCACCTCCC  
GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG

31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCC GGCTGG CCTTAAAAAG  
AGGGTTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTC

31651 CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG  
GTAGTATAGT ACCCATTGTC TGTATAAGAA TCCACAATAT AAGGTGTGCC

31701 TTTCTGTGCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC  
AAAGGACAGC TCGGTTTGCG AGTAGTCACT ATAATTATTT GAGGGGCCCC

31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG  
TCGAGTGAAT TCAAGTACAG CGACAGGTG ACGACTCGGT GTCCGACGAC

31801 TCCAACTTGC GGTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA  
AGGTTGAACG CCAACGAATT GCCCGCCGCT TCCTCTTCAG GTGCGGATGT

31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC  
ACCCCATCT CAGTATTAGC ACGTAGTCCT ATCCCGCCAC CACGACGTGC

31901 AGCGCGCGAA TAACTGCTG CCGCGCCGC TCCGTCCTGC AGGAATACAA  
TCGCGCGCTT ATTTGACGAC GCGCGCGCG AGGCAGGACG TCCTTATGTT

31951 CATGGCAGTG GTCTCCTCAG CGATGATTCG CACCGCCCGC AGCATAAGGC  
GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCCG

32001 GCCTTGTCTT CCGGGCACAG CAGCGCACCC TGATCTCACT TAAATCAGCA  
CGGAACAGGA GGCCCGTGTG GTCGCGTGGG ACTAGAGTGA ATTTAGTCGT

32051 CAGTAACTGC AGCAGAGCAC CACAATATTG TTCAAAATCC CACAGTGCAA  
GTCATTGACG TCGTGTCTGT GTGTTATAAC AAGTTTTAGG GTGTCACGTT

32101 GCGCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGGCCAT  
CCCGACATA GGTTCGAGT ACCGCCCCTG GTGTCTTGGG TGCACCGGTA

32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG  
GTATGGTGTG CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

Figure 27AH

32251 CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC  
GGTATATTTG GAGACTAATT TGTACC GCGG TAGGTGGTGG TAGGATTTGG

32301 AGCTGGCCAA AACCTGCCCC CCGGCTATAC ACTGCAGGGA ACCGGGACTG  
TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC

32351 GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT  
CTTGTTACTG TCACCTCTCG GGTCTGAGC ATTGGTACCT AGTAGTACGA

32401 CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC  
GCAGTACTAT AGTTACAACC GTGTGTGTC CGTGTGCACG TATGTGAAGG

32451 TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGAACAACC  
AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGGT CCCTTGTGG

32501 CATTCCTGAA TCAGCGTAAA TCCCACACTG CAGGGAAGAC CTCGCACGTA  
GTAAGGACTT AGTCGCATTT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT

32551 ACTCACGTTG TGCATTGTCA AAGTGTTACA TTCGGGCAGC AGCGGATGAT  
TGAGTGCAAC ACGTAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA

32601 CCTCCAGTAT GGTAGCGCGG GTTCTGTCT CAAAAGGAGG TAGACGATCC  
GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTTCCTCC ATCTGCTAGG

32651 CTACTGTACG GAGTGC GCGG AGACAACCGA GATCGTGTG GTCGTAGTGT  
GATGACATGC CTCACGCGGC TCTGTTGGCT CTAGCACAAC CAGCATCACA

32701 CATGCCAAAT GGAACGCCGG ACGTAGTCAT ATTTCTGAA GCAAAACCAG  
GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTTGGTC

32751 GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG  
CACGCCCCGA CTGTTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC

32801 CTCTGTGTAG TAGTTGTAGT ATATCCACTC TCTCAAAGCA TCCAGGCGCC  
GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCGCGG

32851 CCCTGGCTTC GGGTTCTATG TAAACTCCTT CATGCGCCGC TGCCCTGATA  
GGGACCGAAG CCAAGATAC ATTTGAGGAA GTACGCGGCG ACGGGACTAT

32901 ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTCTGT  
TGTAGGTGGT GCGTCTTAT TCGGTGTGGG TCGGTGGAT GTGTAAGCAA

32951 CTGCGAGTCA CACACGGGAG GAGCGGGAAG AGCTGGAAGA ACCATGTTTT  
GACGCTCAGT GTGTGCCCTC CTCGCCCTTC TCGACCTTCT TGGTACAAAA

33001 TTTTTTTATT CAAAAGATT ATCCAAAACC TCAAATGAA GATCTATTAA  
AAAAAATAA GGTTTTCTAA TAGGTTTTGG AGTTTTACTT CTAGATAATT

33051 GTGAACGCGC TCCCCTCCGG TGGCGTGGTC AAACCTCTACA GCCAAAGAAC  
CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTTCTTG

33101 AGATAATGGC ATTTGTAAGA TGTTGCACAA TGGCTTCCAA AAGGCAAACG  
TCTATTACCG TAAACATTCT ACAACGTGTT ACCGAAGGTT TTCCGTTTGC

Figure 27 AI

33201 CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC  
GAGATATTTG TAAGGTCGTG GAAAGTTGGTA CGGGTTTATT AAGAGTAGAG

33251 GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCCGGCC  
CGGTGGAAGA GTTATATAGA GATTTCGTTA GGGCTTATAA TTCAGGCCGG

33301 ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG  
TAACATTTTT AGACGAGGTC TCGCGGGAGG TGGAAGTCGG AGTTCGTCCG

33351 AATCATGATT GCAAAAATTC AGGTTCCTCA CAGACCTGTA TAAGATTCAA  
TTAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT

33401 AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTCGCAGGG  
TTCGCCTTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC

33451 CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC  
GGTCGACTTG TATTAGCACG TCCAGACGTG CCTGGTCGCG CCGGTGAAGG

33501 CCGCCAGGAA CCATGACAAA AGAACCACACA CTGATTATGA CACGCATACT  
GGCGGTCCCTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA

33551 CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGGCG  
GCCTCGATAC GATTGGTCGC ATCGGGGCTA CATTCGAACA ACGTACCCGC

33601 GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC  
CGCTATATTT TACGTCCAC GACGAGTTTT TTAGTCCGTT TCGGAGCGCG

33651 AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG  
TTTTTCTTTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATTC

33701 CTCCGGAACC ACCACAGAAA AAGACACCAT TTTCTCTCA AACATGTCTG  
GAGGCCTTGG TGGTGCTTTT TTCTGTGGTA AAAAGAGAGT TTGTACAGAC

33751 CGGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT  
GCCCAAAGAC GTATTTGTGT TTTATTTTAT TGTTTTTTTG TAAATTTGTA

33801 TAGAAGCCTG TCTTACAACA GGAAAAACAA CCTTATAAG CATAAGACGG  
ATCTTCGGAC AGAATGTTGT CCTTTTGTGTT GGGAAATATC GTATTCTGCC

33851 ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA  
TGATGCCGGT ACGGCCGCAC TGGCATTTTT TTGACCAGTG GCACTAATTT

33901 AAGCACCACC GACAGCTCCT CGGTCAATGTC CGGAGTCATA ATGTAAGACT  
TTCGTGGTGG CTGTCGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA

33951 CGGTAAACAC ATCAGGTTGA TTCACATCGG TCAGTGCTAA AAAGCGACCG  
GCCATTTGTG TAGTCCAAC TAAAGTGTAGCC AGTCACGATT TTTGCTGGC

34001 AAATAGCCCG GGGGAATACA TACCCGCAGG CGTAGAGACA ACATTACAGC  
TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAAATGTCG

34051 CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAAACAC  
GGGGTATCCT CCATATTGTT TTAATTATCC TCTCTTTTTG TGTATTTGTG

Figure 27A5



34151 ACATACAGCG CTTCCACAGC GGCAGCCATA ACAGTCAGCC TTACCAGTAA  
TGTATGTCGC GAAGGTGTCG CCGTCGGTAT TGTGAGTCGG AATGGTCATT

34201 AAAAGAAAAC CTATTAAAAA AACACCACTC GACACGGCAC CAGCTCAATC  
TTTTCTTTTG GATAATTTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG

34251 AGTCACAGTG TAAAAAAGGG CCAAGTGCAG AGCGAGTATA TATAGGACTA  
TCAGTGTACAC ATTTTTTCCC GGTTCACGTC TCGCTCATAT ATATCCTGAT

34301 AAAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC  
TTTTTACTGC ATTGCCAATT TCAGGTGTTT TTTGTGGGTC TTTTGGCGTG

34351 GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCTCATA  
CGCTTGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT

34401 TCGTCACTTC CGTTTTCCCA CGTTACGTCA CTTCCCATTT TAAGAAAATC  
AGCAGTGAAG GCAAAAAGGT GCAATGCAGT GAAGGGTAAA ATTCTTTTGA

34451 ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAAACCT ACGTCACCCG  
TGTTAAGGGT TGTGTATGTT CAATGAGGCG GGATTTTGA TGCAGTGGGC

34501 CCCCCTTCCC ACGCCCCGCG CCACGTCACA AACTCCACCC CCTCATTATC  
GGGGCAAGGG TCGGGGCGC GGTGCAGTGT TTGAGGTGGG GGAGTAATAG

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34551 ATATTGGCTT CAATCCAAAA TAAGGTATAT TATTGATGAT GTTAATTIAG  
TATAACCGAA GTTAGGTTTT ATTCCATATA ATAACACTA CAATTAATTC

34601 AATTCGGATC TGCGACGCGA GGCTGGATGG CCTTCCCCAT TATGATTCTT  
TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA

34651 CTCGCTTCCG GCGGCATCGG GATGCCCCGCG TTGCAGGCCA TGCTGTCCAG  
GAGCGAAGGC CGCCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC

34701 GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGQCA  
CGTCCATCTA CTGCTGGTAG TCCCTGTGCA AGTTCCGGTC GTTTTCCGGT

34751 GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC  
CCTTGGCATT TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG

34801 CCTGACGAGC ATCACA AAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC  
GGACTGCTCG TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG

34851 GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC  
CTGTCTTGAT ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCAGC

34901 GCTCTCTCTG TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC  
CGAGAGGACA AGGCTGGGAC GCGGAATGGC CTATGGACAG GCGGAAAGAG

34951 CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG  
GGAAGCCCTT CGCACC CGCA AAGAGTATCG AGTGCACAT CCATAGAGTC

35001 TTCGGTGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG  
AAGCCACATC CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGG

Figure 27 AK

AAGTCGGGCT GGCACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG

35101 CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT  
GGCCATTCTG TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA

35151 TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC  
ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG

35201 CTAAC TACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG  
GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC

35251 AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA  
TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT

35301 AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC  
TTGGTGGCGA CCATCGCCAC CAAAAAACA AACGTTTCGT GTCTAATGCG

35351 GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT  
CGTCTTTTTT TCCTAGAGTT CTCTAGGAA ACTAGAAAAG ATGCCCCAGA

35401 GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT  
CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA

35451 ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA  
TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA TTTAGTTAGA TTTCATATAT

35501 TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA  
ACTCATTGA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT

35551 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTG  
AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCAG

35601 GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC  
CACATCTATT GATGCTATGC CCTCCGAAT GGTAGACCGG GGTCACGACG

35651 AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA  
TTACTATGGC GCTCTGGGTG CGAGTGGCCG AGGTCTAAAT AGTCGTTATT

35701 ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC  
TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG

35751 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC  
CGGAGGTAGG TCAGATAATT AACAACGGCC CTTGATCTC ATTCATCAAG

35801 GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG  
CGGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC

35851 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA  
ACAGTGCGAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT

35901 TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC  
AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG

35951 CTTCCGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC  
GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGCGT CACAATAGTG

Figure 2 AL

36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA  
TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCCTCAACA CGGGATAATA  
CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151 CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT  
GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT  
AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA GGTCAGCTA

36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA  
CATTGGGTGA GCACGTGGGT TGACTIONAG TCGTAGAAAA TGAAAGTGGT

36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA  
CGCAAAGACC CACTCGTTTT TGTCTTCCG TTTTACGGCG TTTTTCCTT

36351 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTCAATA  
TATTCCTGCT GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG  
AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA  
TTACATAAAT CTTTTTATTT GTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAAACCTA  
TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTTCG TCTTCAAGAA TTGGATCCGA  
ATTTTTATCC GCATAGTGCT CCGGGAAAGC AGAAGTTCTT AACCTAGGCT

PacI

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36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)  
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM

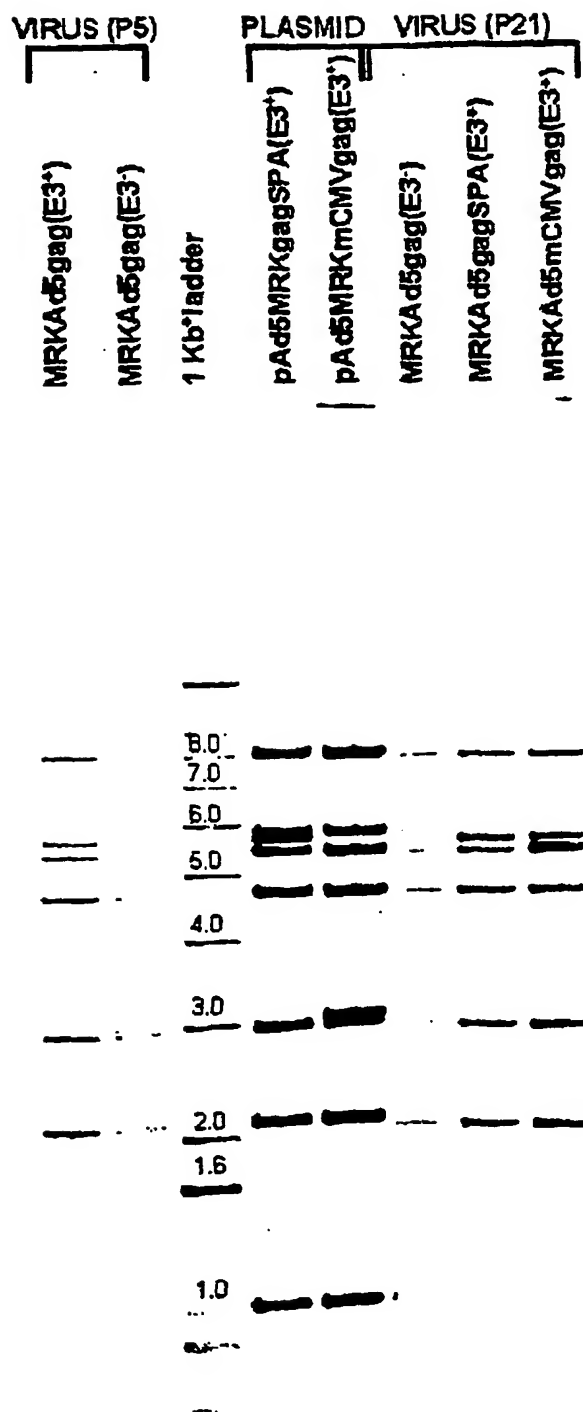


FIGURE 28

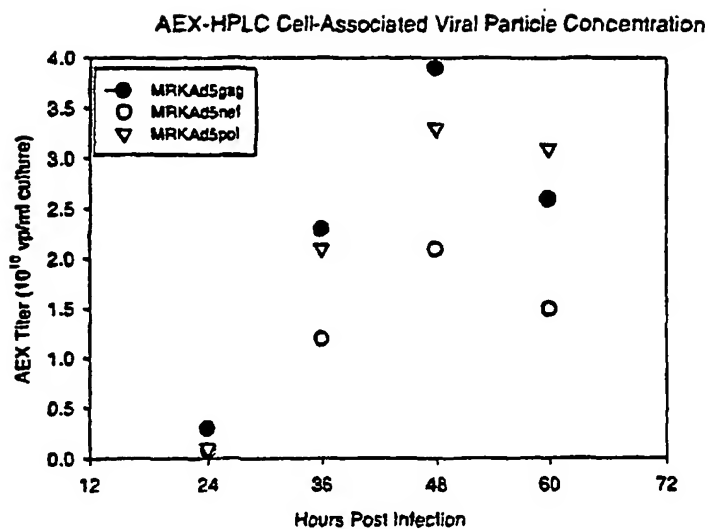


FIGURE 29A

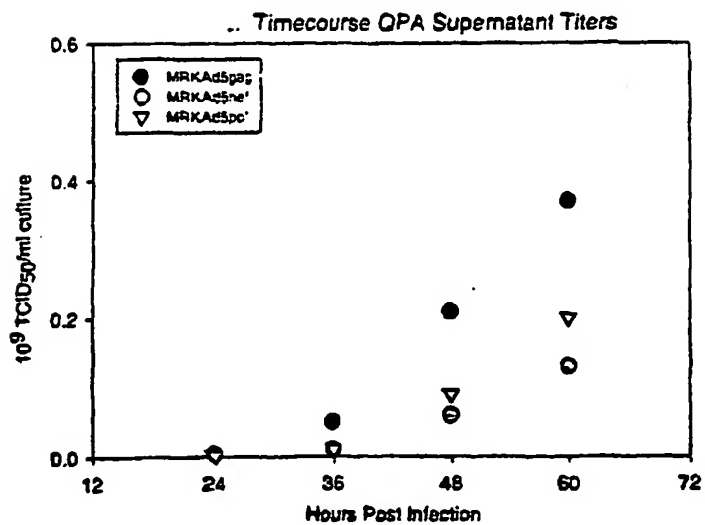


FIGURE 29B

atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga Met Asp Ala Met Lys Arg Gly Leu Cys Val Leu Leu Leu Cys Gly 1 5 10 15	48
gca gtc ttc gtt tgc ccc agc gag atc tcc att gtg tgg gcc tcc agg Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg 20 25 30	96
gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu 35 40 45	144
ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly 50 55 60	192
tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys 65 70 75 80	240
gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys 85 90 95	288
att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala 100 105 110	336
gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val 115 120 125	384
cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr 130 135 140	432
ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu 145 150 155 160	480
gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp 165 170 175	528
ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln 180 185 190	576
atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu 195 200 205	624
cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro 210 215 220	672
agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile 225 230 235 240	720
ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys 245 250 255	768

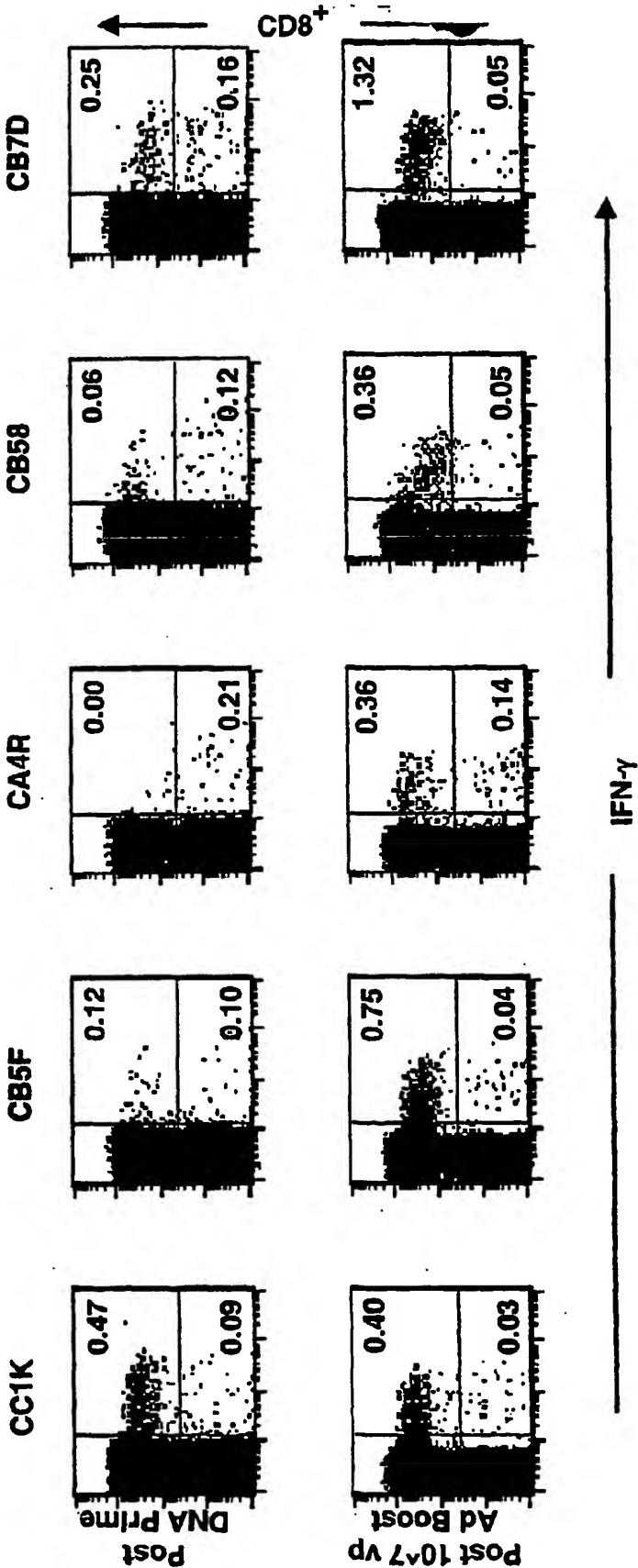
Figure 30A'

agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro 260 265 270	816
acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp 275 280 285	864
tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln 290 295 300	912
gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac Glu Val Lys Asn Trp Met Thr Glu Thr Leu Val Gln Asn Ala Asn 305 310 315 320	960
cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu 325 330 335	1008
gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys 340 345 350	1056
gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr 355 360 365	1104
atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys 370 375 380	1152
tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala 385 390 395 400	1200
ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met 405 410 415	1248
aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro 420 425 430	1296
tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro 435 440 445	1344
aca gcc cct ccc gag gag tcc ttc agg ttt ggg gag gag aag acc acc Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr 450 455 460	1392
ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala 465 470 475 480	1440
tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36) 1482 Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln * (SID NO:37)	
485 490	

Figure 30 B

Figure 31

IFN- $\gamma$  Secretion against Gag 20-aa pool from CD3<sup>+</sup> T cells of Monkey PBMCs





# Comparison of Single-Modality Adenovirus Immunization with DNA+Adjuvant Prime/Adenovirus Boost

## Immunizations

Ad Prime/Boost

DNA-CRL1005 Prime/Ad Boost

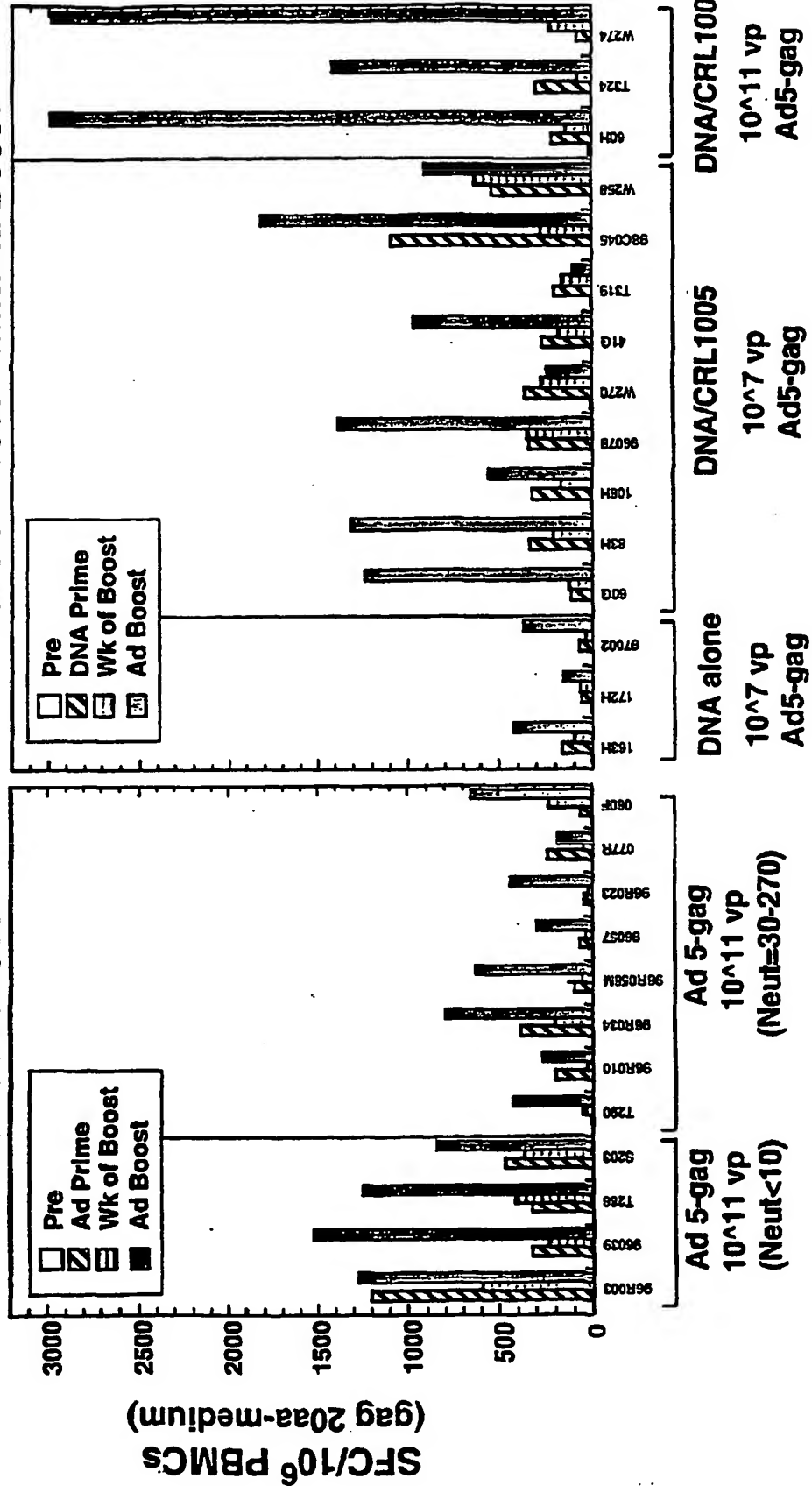


FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG  
 CTGAGGCCTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG  
 CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC  
 CTGGGCCAGC TCCAGCCCTC CCTGCAAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC  
 ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC  
 CTGGAGAAGA TTGAGGAGGA GCAGAACAAG TCCAAGAAGA AGGCCAGCA GGCTGCTGCT  
 GGCACAGGCA ACTCCAGCCA GGTGTCCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC  
 CAGATGGTGC ACCAGGCCAT CTCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG  
 GAGAAGGCCT TCTCCCTTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC  
 CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG  
 CTGAAGGAGA CCATCAATGA GGAGGCTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT  
 GGCCCCATG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC  
 TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACAACC CCCCATCCC TGTGGGGGAA  
 ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAAGATTG TGAGGATGTA CTCCCCACC  
 TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTT  
 TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC  
 CTGCTGGTGC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT  
 GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC  
 AGGGTGCTGG CTGAGGCCAT GTCCCAGGTG ACCAACTCCG CCACCATCAT GATGCAGAGG  
 GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGGCAC  
 ATTGCCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC  
 CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCAACTTCC TGGGCAAAAT CTGGCCCTCC  
 CACAAGGGCA GGCCTGGCAA CTTCCTCCAG TCCAGGCCTG AGCCACAGC CCCTCCCGAG  
 GAGTCCTTCA GGTTTGGGGA GGAGAAGACC ACCCCCAGCC AGAAGCAGGA GCCCATTGAC  
 AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTTG GCAACGACCC CTCTCTCCAG  
 ATGGCTCCCA TCTCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC  
 CCCAAGGTGA AGCAGTGGCC CCTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC  
 ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAAGCC CTACAACACC  
 CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG  
 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC CCACCCCGCT  
 GGCTTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG GGGATGCCTA CTTCTCTGTG  
 CCCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCTCCAT CAACAATGAG  
 ACCCTGGCA TCAGGTACCA GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC  
 ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC  
 AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCCTGAC CACCCCTGAC  
 AAGAAGCACC AGAAGGAGCC CCCCTTCTG TGGATGGGCT ATGAGCTGCA CCCCAGACAAG  
 TGGACTGTGC AGCCCATTTG GCTGCCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG  
 AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG  
 GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG AGCCTGTGCA TGGGGTGTAC

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC  
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG  
GGGGCCACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG  
GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC  
ACCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG  
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG  
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC  
TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG  
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC  
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG  
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC  
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCTT  
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT  
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC  
TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
TCCAACTTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG  
TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG  
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG  
GCTGTGTTCA TCCACAACCT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG  
AGGATTGTGG ACATCATTCG CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT  
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACCTC TGACATCAAG  
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT  
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA

SEQ ID NO: 38

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys  
Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp  
Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser  
Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser  
Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln  
Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln  
Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser  
Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His  
Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys  
Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr  
Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met  
Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His  
Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser  
Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn  
Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu  
Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly  
Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala  
Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln  
Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr  
Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala  
Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met  
Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly  
Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp  
Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn  
Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln  
Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu  
Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu  
Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile  
Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys  
Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys  
Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr  
Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu  
Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu  
Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr  
Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr  
Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met  
Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln  
Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr  
Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp  
Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro  
Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr  
Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu  
Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile  
Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu  
Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr  
Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile  
Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe  
Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile  
Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu  
Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr  
Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp  
Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile  
Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln  
Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu  
Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn  
Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile  
Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val  
Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val  
Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro  
Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp  
Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn  
Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile  
Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val  
Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu  
Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln  
Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu  
Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln  
Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp  
SEQ ID NO: 39

# INTERNATIONAL SEARCH REPORT

International application No.

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## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.	1-3, 8-11, 18 ----- 4, 5, 13-17, 29-32, 34, 35, 37
X --- Y	US 6,019,978 A (ERTL et al.) 1 February 2000 (01/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18 ----- 4, 5, 13-17, 29-32, 34, 35, 37
X,P	US 6,287,571 B1 (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.	1, 9, 18
X --- Y	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18 ----- 4,5,13-17, 29-32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683.	1-3, 9-11, 13-18

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier application or patent published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

06 February 2002 (06.02.2002)

Date of mailing of the international search report

19 AUG 2002

Name and mailing address of the ISA/US

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# INTERNATIONAL SEARCH REPORT

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## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

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## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.



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**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u>

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		and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an <u>HIV Pol protein</u> .
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type

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		adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta</math>E1 and <math>\Delta</math>E3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .
38	86e, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .
39	86f, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
45	86l, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.
48	86o, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

### Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter